The role of interleukin-1β in glucose metabolism during pregnancy and in gestational diabetes mellitus

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Abstract

Pregnancy leads to adaptations of the maternal metabolism and immune system. Increased levels of steroid hormones induce insulin resistance, leading to a glucose gradient from the mother to the fetus, which is modulated by changes in insulin secretion. The maternal adaptive and innate immune systems are modified to accept the fetus, a semi-allograft.

Gestational diabetes mellitus occurs in genetically predisposed women and is associated with obesity and ageing. It is characterized by a further increase of insulin resistance and insufficient insulin secretion. The immune system of women with gestational diabetes is tilted toward inflammation, e.g. with higher interleukin-1β (IL-1β) expression in the adipose tissues and the placenta, and with higher levels of circulating IL-1β.

We investigated the role of IL-1β in glucose metabolism during pregnancy in young chow-fed mice, and older mice fed high-fat diet. To study the effect of IL-1β, a neutralizing anti-IL-1β antibody and IL-1β-deficient mice were used.

Pregnancy impaired glucose tolerance and increased circulating IL-1β and *Il1b* gene expression in the uterus of chow-fed mice and of high-fat diet-fed mice compared to their respective non–pregnant controls. Antagonizing IL-1β improved glucose tolerance of pregnant chow-fed mice and of older high-fat diet-fed mice. Similarly, pregnant IL-1β KO mice showed improved glucose tolerance compared to pregnant littermate control mice, supporting the hypothesis that IL-1β plays a role in pregnancy-induced glucose intolerance. Further, antagonizing IL-1β reduced serum levels of several steroid hormones in healthy pregnant mice. We conclude, that IL-1β contributes to the impairment of glucose metabolism during pregnancy, possibly via modulation of steroid hormones.

Contents

List of abbreviations

AUC	area under the curve
cDNA	complementary deoxyribonucleic acid
CRP	C-reactive protein
C_t	threshold cycle
CYP11B1, CYP11B2	steroid-11β-hydroxylase 1 and 2 or cytochrome P450 11B1 and 2
CYP17A1	steroid 17α-hydroxylase 1 or steroid 17α-monooxygenase or cytochrome P450 17a 1
CYP21A1	steroid 21-hydroxylase or steroid 21-monooxygenase or cytochrome P450 21a 1
GDM	gestational diabetes mellitus
GLUT1, GLUT3	glucose transporter 1, glucose transporter 3
H2-K	H-2 class I histocompatibility antigen, K
HFD	high-fat diet
HLA	human leukocyte antigen
HPA axis	hypothalamic-pituitary-adrenal axis
HSD11B1, HSD11B2	11β-hydroxysteroid dehydrogenase type 1 and 2
HSD3B1 - HSD3B6	3β-hydroxysteroid dehydrogenase/Δ5-isomerase type 1-6
i.p.	intraperitoneal
IL-1β	interleukin-1β
IL-1Ra	interleukin-1 receptor antagonist
IL-6	interleukin-6
ILC3	group 3 innate lymphoid cells
INF-γ	interferon-γ
M2	alternatively activated macrophage
NET	neutrophil extracellular trap
NETosis	neutrophil extracellular trap formation
PBS	phosphate buffered saline
qPCR	quantitative polymerase chain reaction
rcf	relative centrifugal force
RNA	ribonucleic acid
s.c.	subcutaneous
SEM	standard error of the mean
SGLT1	sodium-glucose co-transporter
SRD5A1- SRD5A3	3-oxo-5α-steroid 4-dehydrogenase 1, 2 and 3 or steroid-5α-reductase 1, 2 and 3
T2DM	type 2 diabetes mellitus
TNF-α	tumor necrosis factor-α
uNK-cells	uterine natural killer cells

Introduction

Gestational diabetes mellitus (GDM) is one of the most frequent pregnancy-accompanying diseases. Depending on the population studied and the diagnostic criteria used, it complicates 3-15% of all pregnancies [1, 2]. In older women (45 years and older) the prevalence rises to 26% [2] and in obese women even to 39% [3]. GDM is defined as impairment of glucose tolerance, with or without fasting hyperglycemia, which develops during pregnancy [1]. In most cases, the glucose-tolerance impairment resolves after delivery.

GDM represents a risk for the pregnant woman and for the child:

During pregnancy, all forms of diabetes, including GDM, are a risk factor for preeclampsia [4, 5]. At and around delivery, the following complications can occur: GDM in the mother often causes the child to be large for gestational age or macrosomic (weight at birth ≥ the 90th percentile, for Caucasians ≥ 4000g), which increases the risk for birth complications like shoulder dystocia and the requirement for instrumental deliveries (forceps, caesarean section) [5]. Furthermore, it causes high plasma insulin concentrations in the child. After the circulation of the child is separated from the mother, this may result in a drop of the child's blood glucose, resulting in life threatening hypoglycemia [5]. The risks for jaundice [5], polycythemia [6], hypocalcemia and hypomagnesemia [7] are increased likewise, as well as the risk for respiratory distress syndrome [8].

Depending of the length of the follow up and the ethnicity, 2.6-70% of the women who had GDM later develop type 2 diabetes mellitus (T2DM), with the highest risk within 5 years after delivery [9]. The risk for GDM in a following pregnancy is increased as well [1].

For the child, the lifelong risk to develop obesity, T2DM and GDM is increased [10]. Recently, also childhood asthma [11], atopic dermatitis and early childhood allergen sensitization have been associated to GDM [12].

The pathophysiology of GDM is incompletely understood. One reason for this is probably that our understanding of the glucose metabolism and the special state of the immune system during normal pregnancy is limited.

Glucose metabolism during pregnancy

Glucose is an important nutrient for the developing fetus [13]. Along with free fatty acids and amino acids, glucose is transferred to the fetus through the placenta. Studies in sheep and humans suggest a facilitated passive transport along a glucose gradient [14]. The most important glucose transporter of the placenta is glucose transporter 1 (GLUT1) [15], although also the expression of other glucose transporters has been found, like GLUT3 in human placenta and the sodium/glucose co-transporter (SGLT2) in a human placental cell line [16, 17]. GLUT1 is present in both membranes of the syncytiotrophoblast, that means in both the maternal and the fetal side of the placental barrier. The number of these channels and the size of the syncytiothrophoblast are considered to be rate limiting for glucose transfer to the fetus [15]. However, the placenta doesn't pass on all of the glucose. It is known from humans and rodents, that the placenta can produce and store glycogen [18, 19]. Additionally, large proportions of the glucose that enters the placenta (depending on the gestational age) are used by the placenta itself [20].

The requirement of a glucose gradient from mother to child, in addition to the high glucose need of the fetus, necessitates an increase of maternal hepatic glucose production [21, 22] and a reduction of other glucose sinks in the body of the mother. To achieve this, the maternal body in humans and rodents develops insulin resistance in the insulin target tissues liver, adipose tissue and muscle [23-25]. In the third trimester of human pregnancy, there is a 50% decrease in insulin-stimulated glucose disposal [23]. The metabolism of the mother changes to a higher consumption of fatty acids and ketone bodies to spare the carbohydrates for the fetus [26]. Accordingly, the concentrations of these nutrients along with cholesterol and triglycerides are elevated in the circulation of the mother [27].

It is still a matter of debate how the insulin resistance of the mother develops. From studies in humans and animals it is known that the placenta produces many hormones, which are known to induce insulin resistance, namely cortisol, progesterone, placental lactogen, placental growth hormone and estrogen (at high levels) [24, 28]. The cytokines IL-1β and tumor necrosis factor-α (TNF-α) and several adipokines such as leptin, resistin (both during pregnancy predominantly produced by the placenta) [29] and reduced adiponectin [30] have also been

associated with pregnancy-induced insulin resistance. As mentioned before, the plasma fatty-acid concentration is increased during pregnancy, further adding to direct hormonal effects on insulin sensitivity [13].

Another metabolic adaptation during pregnancy is a 2-2.5 and 3-6-fold increase of insulin secretion in humans [31] and mice [25] respectively. It is needed to maintain euglycemia in face of the insulin resistance. This increased insulin secretion might partly be a direct consequence of the insulin resistance and high blood glucose. However, high blood glucose cannot be the only stimulus for increased insulin secretion during pregnancy, since the glucose gets transferred to the fetus [21, 25]. The increased insulin secretion is achieved by an increase in β-cell mass by hypertrophy and hyperplasia, and a higher insulin output per cell [32]. Many mechanisms are involved in the pregnancy-induced β cell-mass expansion. For example, there are direct proliferation-inducing effects of hormones also implicated in the induction of the insulin resistance, like placental growth hormone and lactogen, glucocorticoids and progesterone. Adiponectin deficiency led to reduced β-cell mass in mice [33-36]. Further, β cells need to down regulate the expression of menin in order to be able to proliferate during pregnancy [37]

Most studies about β cell-mass expansion were conducted in rodents. Therefore, it is important to note that there might be differences in the regulation of β-cell expansion in humans and rodents. One study showed, that in contrast to rodents, β-cell expansion during human pregnancy is primarily achieved through the formation of new pancreatic islets arising from islet-cell progenitors such as ductal cells, instead of proliferation of existing β cells [38, 39].

Immunological changes during pregnancy

The fetus and the placenta are a semi-allograft in the body of the mother. This means that the fetal-placental unit has to avoid rejection [40]. To maintain the pregnancy with simultaneous maintenance of immunological protection, the systemic innate and adaptive immune systems of the mother have to adapt. The lymphocyte populations are decreased, but the monocyte and neutrophil counts are increased, resulting in higher total leukocyte counts [41, 42].

Additionally, there is a highly specialized immune environment created at the fetal-maternal interface:

Placentas of primates and rodents are hemochorial, which means that fetal trophoblast cells (cells of the placenta) are in direct contact with the maternal blood. In both, primates and rodents, trophoblast cells also invade the uterine wall and the uterine blood vessels at the implantation site of the placenta, the decidua [43]. Except for human leukocyte antigen-C (HLA-C) in humans and H-2 class I histocompatibility antigen, K (H2-K) in mice, the invading cells don't express classical major histocompatibility complex (MHC) class I molecules and are therefore barely antigenic. Human invading trophoblast cells express HLA-G and HLA-E instead. The not invading villous trophoblast cells completely lack MHC-complex expression in humans [44, 45].

Many specialized immune cells are located in the decidua. Their composition changes with the progress of the pregnancy, and their function is not fully understood.

During the first trimester, uterine natural killer cells (uNK-cells) are with 70-80% of total immune cells the most abundant immune cell types of the decidua in mice and human [46]. They have important roles in the decidualization, the trophoblast invasion and the remodeling of the spiral arteries. Their cytotoxic function is inhibited, but they are part of the complex immune-modulating cytokine network and express IL-1β, TNF-α, interleukin-6 (IL-6) and interferon-γ (IFN-γ) and other cytokines [47]. In the middle of the pregnancy, the number of uNK-cells starts to decline and reverts back to pre-pregnancy levels in the third trimester.

In the third trimester of pregnancy, myeloid cells are the most abundant immune cells in the decidua of mice, including neutrophils, monocytes, dendritic cells and macrophages [48].

Like the uNK-cells, the decidual macrophages decrease in number towards the end of pregnancy. Nevertheless, they are with 20-30% abundant decidual immune cells throughout pregnancy [49]. Several macrophage subpopulations with distinct functions have been identified in the decidua. The largest subset has an M2-like phenotype, responsible for phagocytosis and the removal of cell debris, but also the presence of a more proinflammatory subset has been reported [50].

Dendritic cells in the decidua are of myeloid origin. They are relatively rare but important, inducing T-regulatory cells, recruiting NK-cells and regulating angiogenesis. Ablation of decidual dendritic cells in mice led to increased abortion rates [44]. Another study done in mice showed that, in contrast to other dendritic cells, decidual dendritic cells are entrapped in the decidua, are not able to present antigens in lymph nodes and do not activate T-cells [51].

There is not much known about the role of neutrophils in the decidua, although they are an abundant resident cell type, especially in the second trimester. Decidual neutrophils are characterized by high levels of activation markers and the expression of proteins related to angiogenesis in both, mice and humans [52]. Recently, it has been shown that group 3 innate lymphoid cells (ILC-3) recruit neutrophils to the decidua [53].

Steroid hormones and insulin resistance

Placental steroid hormones contribute to pregnancy-induced insulin resistance [28]. However, the insulin-desensitizing effect of glucocorticoids and sex steroids are not restricted to pregnancy. Progesterone and estradiol influence insulin sensitivity of women during the menstrual cycle [54]. Women with polycystic ovary syndrome (a state of hyperandrogenemia) suffer from insulin resistance [55]. In contrast to this, the sex hormones testosterone and estrogen (at lower levels than during pregnancy) prevent mice from becoming insulin resistant [56, 57]. Testosterone replacement therapy improved insulin sensitivity in hypogonadal men with T2DM [58].

Patients with chronically increased cortisol levels (Cushing syndrome) suffer from insulin resistance [59]. Together with catecholamines and cytokines, glucocorticoids reduce insulin sensitivity in obesity and psychiatric stress [60]. Corticosterone, the most important glucocorticoid in rodents [61], induces insulin resistance in mice [62]. Male leptin-resistant, highly insulin resistant db/db mice have increased plasma levels of aldosterone, corticosterone, 11-deoxycorticosterone and progesterone, along with increased gene expression of the enzymes involved in the synthesis of these steroid hormones in the adipose tissue [63].

Interestingly, IL-1β has been shown to stimulate the production of various steroid hormones: It stimulates the production of progesterone from a human placental cell line [64] and bovine granulosa cells (cells in ovarian follicles) [65]. In rodents, IL-1β stimulates the increase of serum corticosterone by influencing the hypothalamic-pituitary-adrenal axis (HPA axis) on hypothalamic, pituitary and adrenal level [66-69]. In humans, the administration of IL-1Ra decreases serum cortisol in obese individuals [70].

Pathophysiology of gestational diabetes

In GDM, many parts of the metabolic and immunological adaptation to pregnancy do not work properly. Metabolically, GDM is characterized by a further increase of insulin resistance with an inadequate insulin response by the pancreatic islets. Although the islets of women with GDM secrete more insulin than that of women with healthy pregnancies, it is not enough to overcome the insulin resistance [31, 71]. It is not known if β-cell mass is altered in women with GDM compared to women with healthy pregnancies, but mouse models, in which the β-cell proliferation was hampered, led to GDM [33, 36, 37, 72].

At a cellular level, GDM is associated with impaired insulin signaling in muscle cells, hepatocytes and adipocytes. This results in a 65% reduced glucose disposal compared to non-pregnant women, increased lipolysis and increased hepatic glucose production [21, 73].

The release of leptin from the placenta is decreased in GDM, but the release from adipose tissue is increased compared to healthy pregnant women [29].

One of the most important risk factors for GDM is obesity. Obese women have more inflammatory macrophages accumulating in the placenta, which produce cytokines like IL-1, TNF-α and IL-6, compared to lean pregnant women [74]. TNF-α, IL-6 and C-reactive protein (CRP) are increased in the circulation of women with GDM, as well as and the expression of TNF-α and IL-6 in the adipose tissue [75]. Among various other effects, the increased TNF-α in the circulation leads to increased formation of neutrophil extracellular traps (NETosis) of peripheral neutrophils in women with GDM [76].

IL-1β is also increased in the serum of women with GDM, and in obese pregnant women [77, 78]. In the adipose tissue of women with GDM, the inflammasomes are induced [79], presumably by high concentrations of free fatty acids and increased endoplasmatic reticulum stress [80], leading to IL-1β secretion.

Obesity and GDM are associated with decreased circulating adiponectin [30, 77]. Adiponectin-deficient mice develop GDM with increased insulin resistance, increased hepatic glucose production and decreased β-cell mass during pregnancy [36].

Until recently the placenta and decidua were thought to be sterile. This view changed with the discovery of a placental microbiome, which is distinct from other microbiota of the human body [81]. In the meantime, it has been shown that GDM and excessive weight gain during pregnancy are accompanied by changes in this microbiome [82, 83]. Further, the gut microbiome is also altered in women with GDM [84].

IL-1β and type 2 diabetes

IL-1β was the first cytokine to be discovered. In the 1970s, it has been described as the factor causing fever and as a mitogen for T-cells [85, 86]. Today, it is known as a master regulator of inflammation with multiple functions in physiology and pathophysiology [87, 88].

In the last 20 years, it has become evident that obesity-induced insulin resistance is mediated through inflammatory factors, including IL-1β [89, 90]. In obese subjects and patients with T2DM, IL-1β secretion, presumably from tissue resident macrophages [91], is increased in insulin-sensitive tissues.

Furthermore, it has been shown that IL-1β is upregulated in pancreatic islets of patients with T2DM and that IL-1β contributes to β-cell glucotoxicity and lipotoxicity, to β-cell destruction and dedifferentiation [92-96].

Since IL-1β causes insulin resistance and β-cell failure, and has furthermore a role in the cardiovascular complications of diabetes [97] it is an attractive therapeutic target [98]. In a clinical study, IL-1 antagonism using the IL-1 receptor antagonist (IL-1Ra) improved gylcemia and insulin secretion in patients with T2DM [99].

Currently, the CANTOS trial, a phase III clinical study with more than 10000 participants, investigates if Canakinumab, a neutralizing monoclonal anti-IL-1β antibody is effective in reducing the incidence and influencing the course of T2DM [100].

Aim of this study

Since IL-1β is increased in women with GDM and has a role in T2DM, and since IL-1β antagonism improves β-cell function and insulin sensitivity in diabetic subjects [99, 101] we aim to study the effect of IL-1β antagonism on glucose metabolism during pregnancy of normal mice and in a mouse model of GDM.

Materials and Methods

Mice:

All mouse experiments were approved by the animal-welfare committee of the Kanton Basel Stadt. Mice were either purchased from Charles River (Sulzfeld, Germany) or originated from our in-house breeding. For the experiments with wild-type mice we used C57BL/6N mice, all transgenic mice were on a C57BL/6N background. All experiments with transgenic mice were done with littermate controls.

We used two transgenic mouse models. A constitutive, whole-body IL-1β-deficient mouse strain (IL-1β KO) and a myeloid lineage-specific IL-1β-deficient mouse: the *Il1b*$^{fl/fl}$*Lyz2*-Cre mouse (LysMCre-IL-1β KO) [88]. The work with the LysMCre-IL-1β KO mouse was performed together with Valmir Makshana.

Female mice of different ages were timed-mated. Used bedding of cages of males was put into the cages of the females to align their menstrual cycle and induce their estrus. 2.5 days later, the females were transferred into the cage of male breeders for 24h, before returning to their own cage. The day of plug detection was regarded as day 0.5 of pregnancy.

If indicated, mice received one single i.p. injection of a murine anti-IL-1β antibody (BSUR05, with the same specificity as canakinumab; kindly provided by Novartis, Switzerland), or vehicle on day 7.5 of pregnancy. Metabolic testing of the mice followed on day 13.5 of pregnancy and the sacrifice on day 14.5 of pregnancy.

If indicated, mice received a high-fat diet with 60 kJ% fat (lard; ssniff Spezialitäten, Soest, Germany), all other mice received normal mouse chow diet (Kliba, Kaiseraugst, Switzerland). If not indicated differently, all mice had free access to food and water and were housed in a 12-hours light and dark cycle with the light phase during the day.

All experiments were repeated with at least three independent cohorts, if not indicated otherwise.

Metabolic testing:

Glucose tolerance testing: For glucose tolerance tests, mice were fasted for 6 hours in the morning. The fasted mice received a subcutaneous bolus injection of 2 g glucose per kg body weight. Blood glucose was measured prior to, and 15, 30, 60, 90 and 120 minutes after the injection, each with a drop of capillary blood from the tail tip, using FreeStyle Lite glucose meters (Abbott AG, Baar, Switzerland). At the first three time points, additional blood samples were taken from the tail tip into tubes containing EDTA to measure plasma insulin with the MSD Mouse/Rat Insulin Kit (Mesoscale Discovery, Rockville MD, USA) according to the instructions of the manufacturer. The insulinogenic index was calculated as follows:

$$insulinogenic\ index = \frac{(plasma\ insulin\ 15' - basal\ plasma\ insulin)}{(blood\ glucose\ 15' - basal\ plasma\ insulin)}$$

Insulin tolerance testing: Mice were fasted for three hours in the morning. The fasted mice received a subcutaneous injection of 0.01 IU insulin (Actrapid, Novo Nordisk, Copenhagen, Denmark) and blood glucose was measured as described for the glucose tolerance testing.

Serum preparation:

Serum was obtained by allowing fresh blood to stand for 30 minutes at room temperature, followed by centrifugation at 2000 rcf for 20 minutes.

Serum IL-1β measurements:

Serum-IL-1β measurements were performed with the MSD mouse IL-1β Kit (Mesoscale Discovery) according to the "alternative protocol 2" of the manufacturer's instructions.

Serum hormone measurements:

Serum steroid hormones were quantified using ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) as described in [102]. The measurements were performed by Denise Kratschmar, University of Basel, Department of Pharmaceutical Sciences, Klingelbergstrasse 50, 4056 Basel.

Ribonucleic acid (RNA) extraction and quantitative polymerase chain reaction (qPCR):

Islets and whole-blood cells were directly lysed, tissue was homogenized with an electrical Polytron homogenizer (Kinematica, Lucerne, Switzerland) in lysis buffer of the RNA extraction kit. RNA was isolated using the NucleoSpin RNA II Kit (Macherey Nagel, Düren, Germany) according to the instructions of the manufacturer. Complementary deoxyribonucleic acid (cDNA) was prepared using the deoyxnucleoside triphosphate (dNTP) set (Roche Diagnostics, Indianapolis, IN, USA), 1OD 260 hexamer (Mircrosynth, Balgach Switzerland) and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and used for qPCR. For qPCR we used GoTaq polymerase mixes (GoTaq Probe qPCR Master mix for Taqman probes and GoTaq qPCR Mastermix for the SYBRgreen method; both Promega, Catalys, Switzerland) and the following ABI Taqman probes (ThermofisherScientific, Reinach, Switzerland): *Il1b*: Mm00434228, *Gapdh*: Mm99999915, *Actinb*: Mm00607939, *18s*: Hs99999901_s1, *Mki67*: Mm01278617, *Ccna2*: Mm00438063, *Pcna*: Mm00448100_g1. The primers (Microsynth) used for SYBR green detection are listed in table 1.

Table 1:

target	direction	primer
Gapdh	forward	AGGTCGGTGTGAACGGATTTG
	reverse	TGTAGACCATGTAGTTGAGGTCA
Actinb	forward	GGCTGTATTCCCCTCCATCG
	reverse	CCAGTTGGTAACAATGCCATGT
Cyp11b1	forward	AGAGCTGGTAGCTGAGAGAAC
	reverse	CCTTCTGAGGATTTGCAGCGA
Cyp11b2	forward	CGTGGCCTGAGACGTGGTGT
	reverse	CATCCATGGTAAGGCTCCCACGA
Cyp21a1	forward	TCCCCTTTCTCAGGTTCCTCC
	reverse	CTTTCCATTGGCCTGCAACC

Mouse islet isolation:

Islets were isolated using collagenase digestion (4189, Worthington, Lakewood, NJ, USA). The pancreas was perfused with collagenase solution in situ, dissected and incubated for 28 min at 37°C in a water bath. Subsequently, the islets were washed, filtered with a 70µm cell strainer (Corning, Durham, NC, USA) and hand-picked [103].

β-Cell area determination:

Mouse pancreata were dissected and fixed in 4% formalin (Hittnau, Switzerland) over night, washed in phosphate buffered saline (PBS) and embedded in paraffin. Of each pancreas, serial sections of 5 µm were taken every 100 µm trough the pancreas. This resulted in 6-13 sections per pancreas. The sections were stained after heat-induced antigen retrieval in Target Retrieval Solution (pH=6, Dako, Aligent Technologies, Santa Clara, CA, USA). The following antibodies were used for staining: primary: polyclonal guinea pig-anti-insulin (Dako; 1:200); secondary: Fluorescein (FITC)-conjugated donkey anti-guinea pig (Jackson ImmunoResearch, 1:200). Nuclei were stained with DAPI, dilactate (Sigma, 1:100000) added to the secondary antibody.

Insulin was stained to determine the area of β cells on each section. The DAPI-signal was used to determine the total pancreatic area of the section. β-cell area is presented as insulin-positive area divided by the total pancreas area. Microscopy was performed with an Olympus IX83 microscope. For picture analysis, the CellSence software (Olympus Schweiz AG, Volketswil, Switzerland) was used.

Graphs and statistics

For graphs and statistics we used Prism 7 software (Graph Pad, La Jolla, CA, USA). Outliers, identified with the ROUT test, were excluded with a sample size of above 5. The statistical test used in each study is noted in the corresponding figure legend.

Results

A mouse model of gestational diabetes

We tested if GDM can be induced in C57BL/6N mice by applying two important risk factors for GDM: age and obesity. Therefore, mice were mated at different ages and fed either normal chow or a high-fat diet. Mice, which didn't become pregnant, served as controls. We measured fasting glucose and glucose tolerance on day 13.5 of pregnancy, which is at the beginning of the third trimester of a mouse pregnancy. The glucose tolerance was tested using subcutaneous glucose tolerance tests, in which the mice received a subcutaneous glucose-bolus injection after a fasting period of 6 hours. The insulinogenic index is the quotient of the insulin increment (Δ insulin) and the glucose increment (Δ glucose) 15 minutes after the glucose bolus injection.

Pregnant young (10-13 weeks old) chow-fed mice had mildly impaired glucose tolerance with mildly increased fasting glucose compared to non-pregnant controls. Their plasma insulin was increased basally and throughout the test. The insulinogenic index in pregnant and control mice was not significantly different (Fig. 1A, E, I, M).
Older (15-18 weeks old) chow-fed mice had a similar glucose phenotype (Fig. 1B, F, J, N). These models will further be referred to as "healthy pregnant".

To test if high-fat diet feeding during pregnancy would be sufficient to cause GDM, chow-fed mice were switched to high-fat-diet feeding after timed mating. The controls received high-fat diet during this time, as well. This resulted in two weeks of high-fat-diet feeding before the metabolic testing at 16 weeks of age. The glucose tolerance of both, pregnant and control mice, was impaired compared to that of mice of the same age fed chow diet, along with increased plasma insulin levels. The impairment of glucose tolerance and the increase of plasma insulin were more pronounced in the pregnant mice, both blood glucose and plasma insulin were increased compared to the controls. Fasting blood glucose and insulinogenic index were similar and comparable to that of the

Figure 1

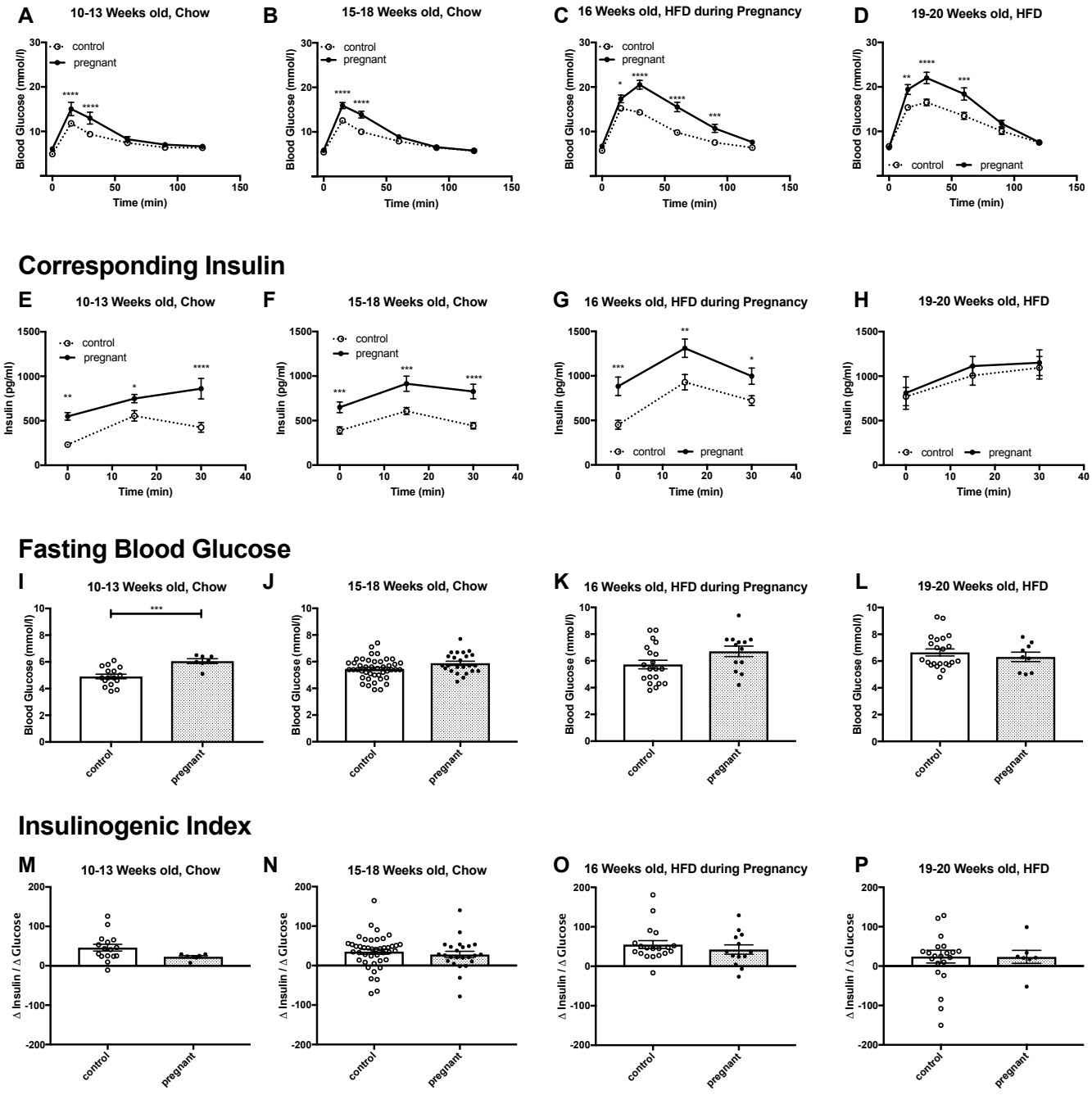


Figure 1

Glucose tolerance (**A**-**D**), plasma insulin (**E**-**H**), fasting blood glucose (**I**-**L**) and insulinogenic index (**M**-**P**) of pregnant mice on day 13.5 of pregnancy: **A**, **E**, **I**, **M**: 10-13 weeks old mice fed normal chow diet: control n=16, pregnant n=7; **B**, **F**, **J**, **N**: 15-18 weeks old mice fed normal chow: control n=44, pregnant n=24; **C**, **G**, **K**, **O**: 16 weeks old mice fed chow before pregnancy, but HFD during pregnancy: control n=20, pregnant n=13; **D**, **H**, **L**, **P**: 19-21 weeks old mice fed HFD for 3-6 weeks before mating and during pregnancy, control n=22, pregnant n=9; all data as mean ± SEM, statistics in A-H: 2way ANOVA for repeated measurements with Holm-Sidak multiple comparisons, statistics in I-P: Mann-Whitney test.

chow-fed healthy pregnant mice (Fig. 1C, G, K, O). This model will be referred to as “short-term high fat diet”.

Ultimately, mice were fed high-fat diet 3-6 weeks before timed mating at the age of 17-18 weeks and throughout pregnancy, resulting in 5-8 weeks of high-fat-diet feeding on the day of the glucose tolerance tests at the age of 19-21 weeks. Controls received the same diet. In these tests, pregnant mice had a marked impairment of glucose tolerance compared to the controls. The controls also showed an impairment of glucose tolerance compared to the controls in the healthy pregnant and short-term high-fat diet groups. The insulin secretion of pregnant mice and controls was similar. Nevertheless, the insulinogenic index showed no difference. The fasting glucose was the same (Fig. 1D, H, L, P). This model will further be referred to as “GDM model”.

Taking into account all pregnant mice from all models described above, the area under the curve (AUC) of the glucose tolerance tests of pregnant mice positively correlated with the weight of the mice before pregnancy (Fig. 2A).

Mice fed a high-fat diet tended to be more fertile then mice fed chow diet. The percentage of pregnant mice after timed-mating was higher in females that received high-fat diet. This difference was statistically not significant (Fig. 2B).

Figure 2

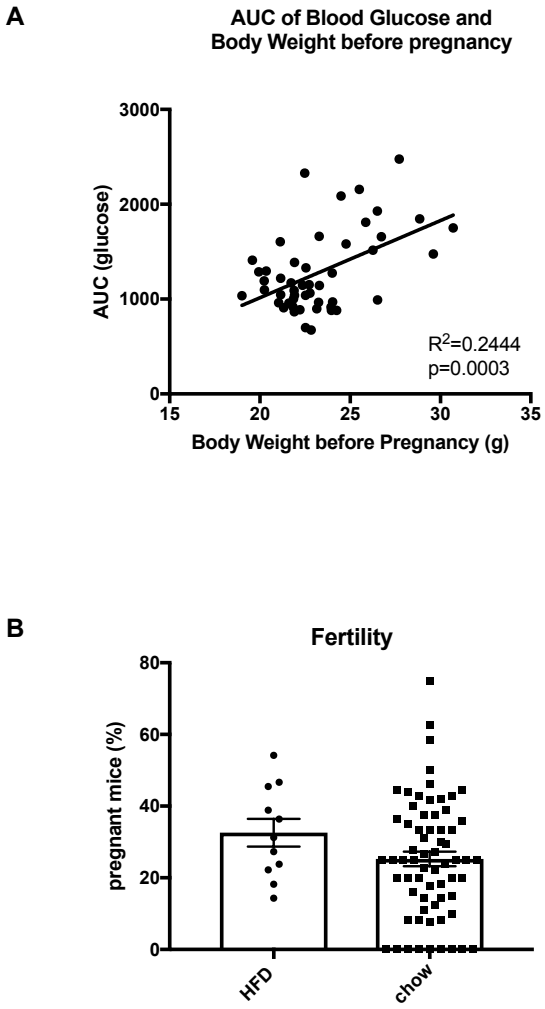


Figure 2
A: Area under the curve of the glucose tolerance tests and the body weight before pregnancy of all pregnant mice from figure 1 n=49, with linear regression; **B:** Fertility expressed as % of mice which became pregnant at timed-mating: HFD: n=11, chow: n=66

A known risk of GDM in humans is macrosomia of the child. Therefore, we measured the fetal weight of healthy pregnant mice, short-term high-fat diet mice and GDM mice at sacrifice (Fig. 3). Older healthy pregnant mice had on average smaller fetuses than younger healthy pregnant mice, whereas the average fetal weight of short-term high-fat diet mice and GDM mice was not different (Fig. 3A). The total fetal weight (the sum of the weight of all fetuses from one pregnant mouse; Fig. 3B) and the litter size (the number of pubs per pregnant mouse; Fig. 3C) were not dependent on age or diet of the pregnant mouse.

Figure 3: Fetal Weight

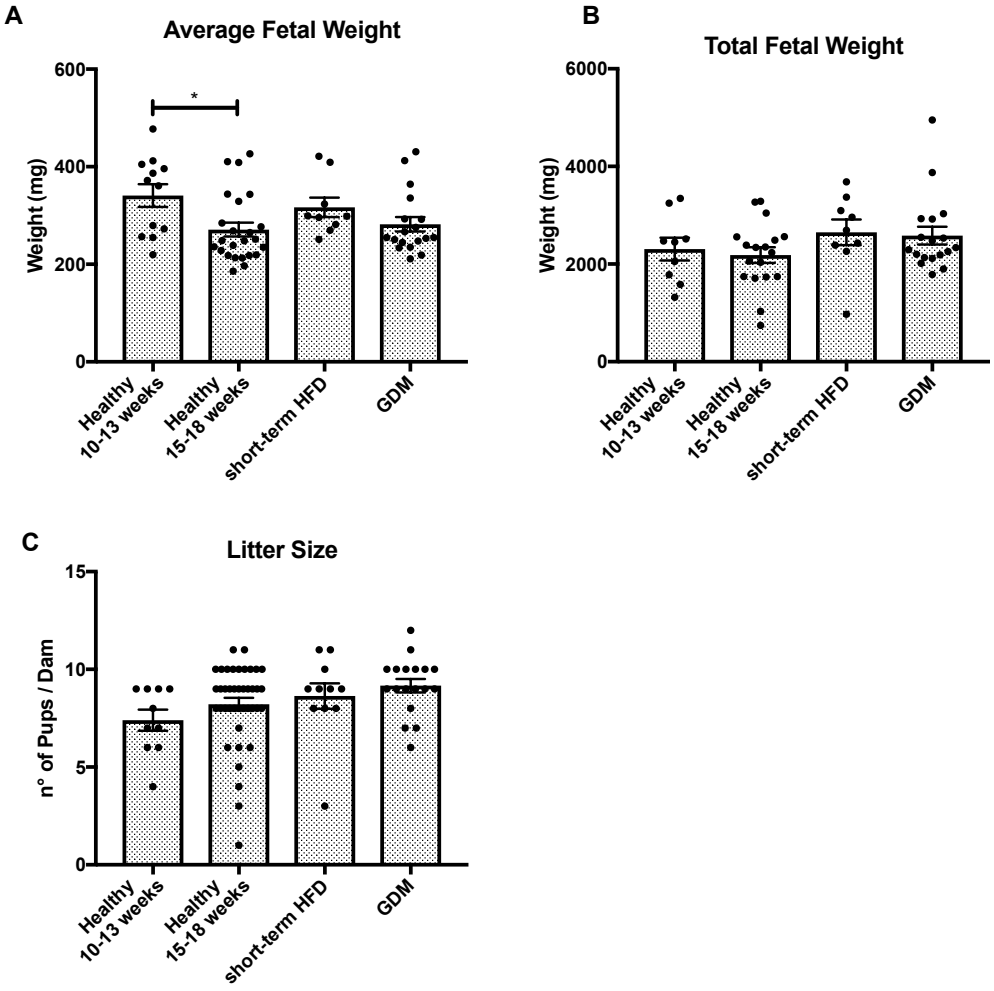


Figure 3
A-C: Average fetal weight, tolal litter weight and litter size, respectively, in all pregnant mice from figure 1, healthy 10-13 weeks n=9, healthy 15-18 weeks n=18, short-term HFD n=9, GDM n=18; data as mean ± SEM, statistics: Kruskal-Wallis test with Dunn's multiple comparison.

To examine if GDM mice have increased IL-1β levels, we measured IL-1β in the serum of our mouse models under two conditions: In 6-hours fasted mice in the afternoon (at 2 p.m.; Fig. 4A and C) and in not fasted mice in the afternoon (at 3 p.m.; Fig. 4B and D). The serum IL-1β did not differ in fasted mice, although the serum IL-1β of mice in the GDM model was higher than that in the healthy pregnant model (Fig. 4A and B).

However, not fasted pregnant mice had higher serum IL-1β levels than the controls, both in the healthy pregnant model and in the short-term high-fat diet model (Fig. 4C and D). Unfortunately, the sample size in the not fasted condition was small and we have no sera from not fasted GDM mice.

Figure 4: Serum IL-1β

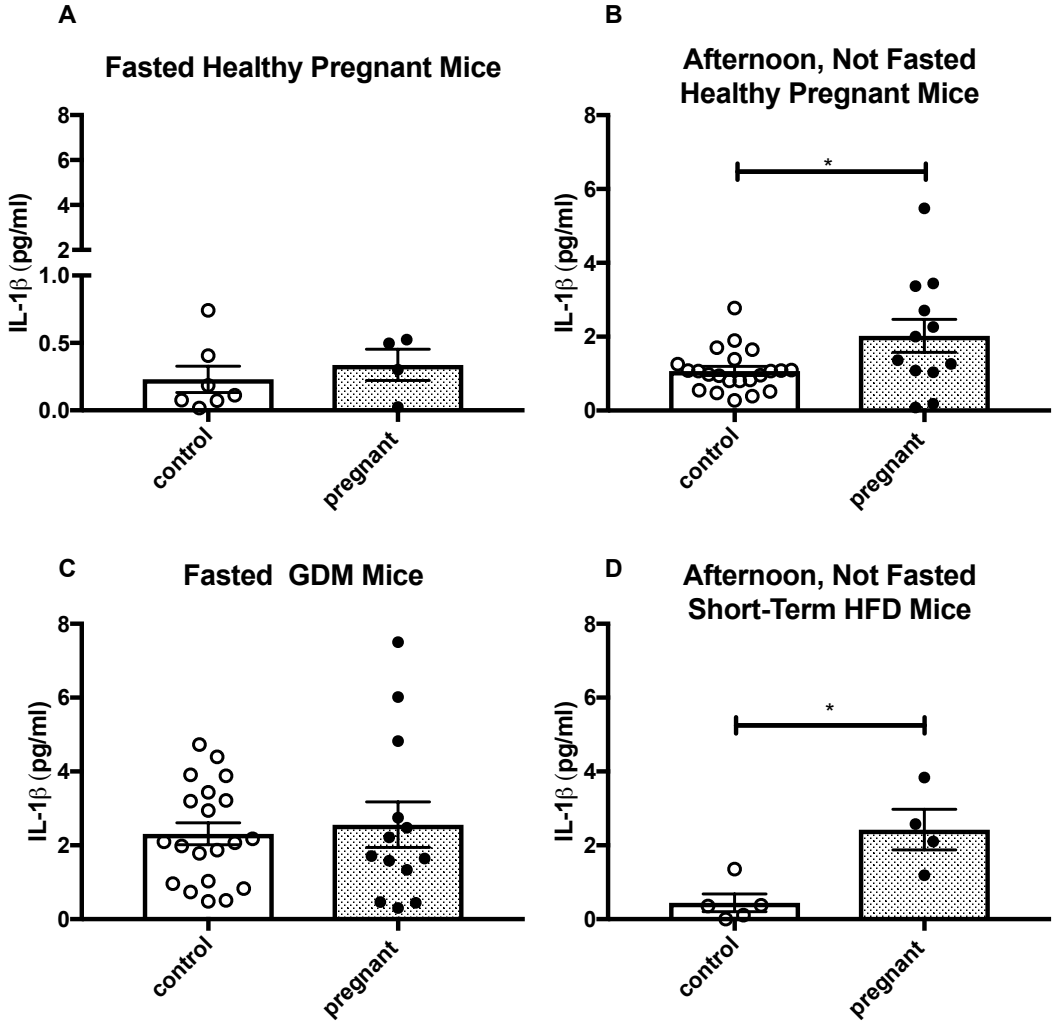


Figure 4
Serum IL-1β of **A:** Fasted healthy pregnant mice: control n=7, pregnant n=4; **B**: Not fasted healthy pregnant mice: control n=22, pregnant n=12; **C**: Fasted GDM mice: control n=20, pregnant n=13; **D**: Not fasted short-term HFD mice: control n=5, pregnant n=4; data as mean ± SEM, statistics with Mann-Whitney test

Organ/tissue *Il1b* gene expression in pregnant mice

By measuring the *Il1b* gene expression in candidate organs and tissues, we tried to localize the source of increased IL-1β in pregnant mice that might influence the glucose tolerance (Fig. 5). We isolated RNA from pieces of tissues, from peripheral blood cells and from isolated islets and tested if *Il1b* gene expression was altered during healthy pregnancy or in GDM. All samples were collected on day 14.5 of pregnancy.

Figure 5: *ILb* Gene Expression

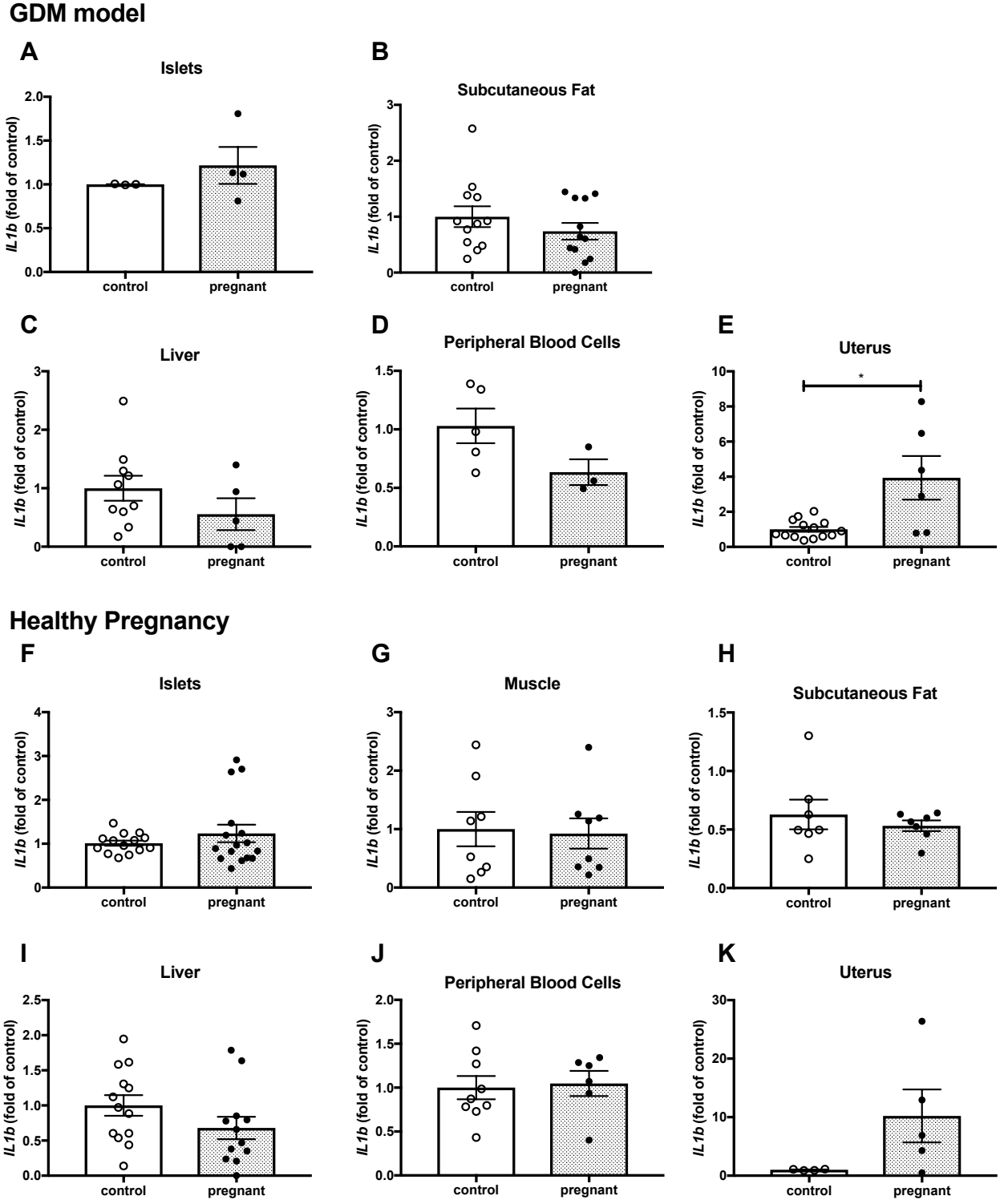


Figure 5

IL1b gene expression in candidate organs/ tissues in pregant mice and not pregnant controls. **A-E**: *IL1b* gene expression in GDM mice **A**: Islets: control n=3, pregnant n=4; **B**: Subcutaneous fat: control n=12, pregnant n=12; **C**: Liver: control n=10, pregnant n=5; **D**: Peripheral blood cells control n=5, pregnant n=3; **E**: Uterus controls n=6, pregnant n=14;
F-K: *IL1b* gene expression in healthy pregnant mice. **F**: Islets: control n=14, pregnant n=16; **G**: Muscle: control n=8, pregnant n=8; **H**: Subcutaneous fat: control n=7, pregnant n=7; **I**: Liver: control n=13, pregnant n=12; **J**: Peripheral blood cells: control n=9, pregnant n=7; **K**: Uterus: control n=4, pregnant n=5; data expressed as mean ± SEM; statistics: Man-Whitney test, note the different scales of the y-axes.

Figure 6: Reference Gene Expression in Uterus

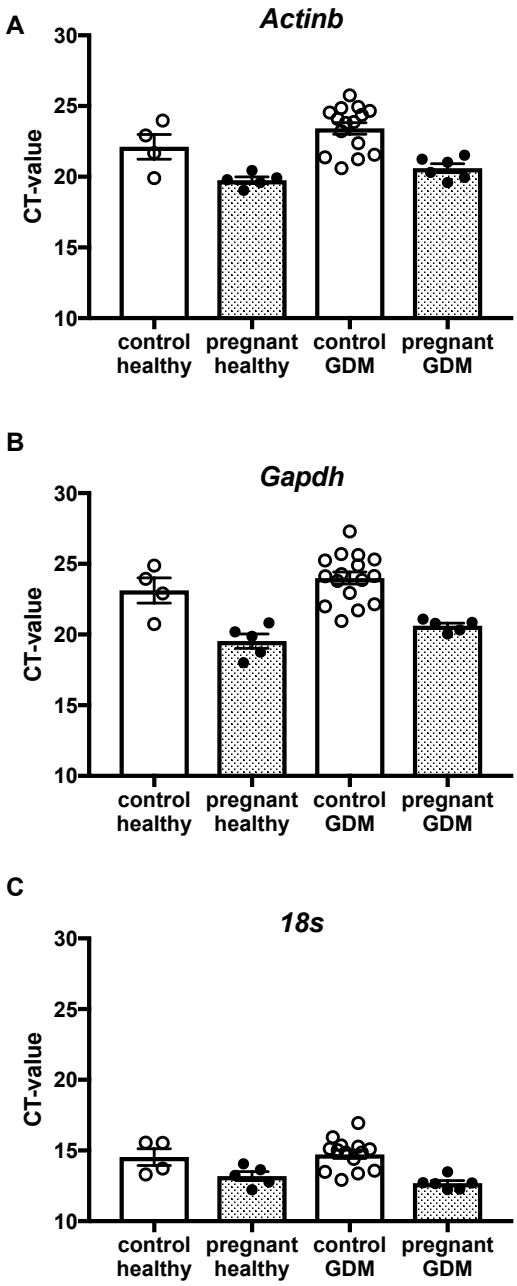


Figure 6
qPCR results expressed as ct-values for the reference genes *Actinb* (**A**), *Gapdh* (**B**) and *18s* (**C**); healthy control: n=4, healthy pregnant: n=5, GDM control: n=15, pregnant GDM: n=6; data as mean ± SEM

There was no difference of *Il1b* gene expression in islets and the subcutaneous adipose tissue of pregnant mice of the GDM model (Fig. 5A, B), and in islets, muscle, subcutaneous adipose tissue and peripheral blood cells of healthy pregnant mice compared to non-pregnant controls (Fig. 5F, G, H, J).

In GDM mice, there was a trend for lower *Il1b* expression in the liver (Fig 5C) and peripheral blood cells (Fig. 5D). In healthy pregnant mice, there was a trend for lower *Il1b* gene expression in the liver as well (Fig 5I).

Il1b expression in the uterus of pregnant mice was increased in GDM mice (Fig. 5E) and tended to be increased in healthy pregnancy (Fig. 5K). To extract RNA from the uterus, we only took the uterus wall with the decidua. We dissected off the placenta (the RNA of the placenta was analyzed separately), fetus and amnion. In the uterus, not only the expression of *Il1b* was increased, but also the expression of the reference genes *Actinb* and *Gapdh,* despite the same RNA input to the reverse transcription (Fig. 6A and B). Thus, data for this organ were normalized to the expression of *18s,* which was more stable than *Actinb* and *Gapdh* (Fig. 6C).

Inhibition of IL-1β in GDM mice and healthy pregnant mice

Hypothesizing that IL-1β contributes to the impaired glucose tolerance of pregnant mice with GDM, we inhibited IL-1β using a murine neutralizing anti-IL-1β antibody (anti-IL-1β) with the same specificity as canakinumab. Anti-IL-1β was administered as single dose with an i.p. injection on day 7.5 of pregnancy, which is at the beginning of the second trimester.

Anti-IL-1β treatment improved the glucose tolerance of pregnant GDM mice (Fig. 7A). Although not significant, there was a trend for higher glucose-stimulated insulin secretion in pregnant GDM mice treated with anti-IL-1β (Fig. 7B), but not in the non-pregnant control mice. Nevertheless, the insulinogenic index tended to be improved in both pregnant and non-pregnant mice (Fig. 7D). The fasting blood glucose was not affected by anti-IL-1β (Fig. 7C).

Figure 7

GDM Model

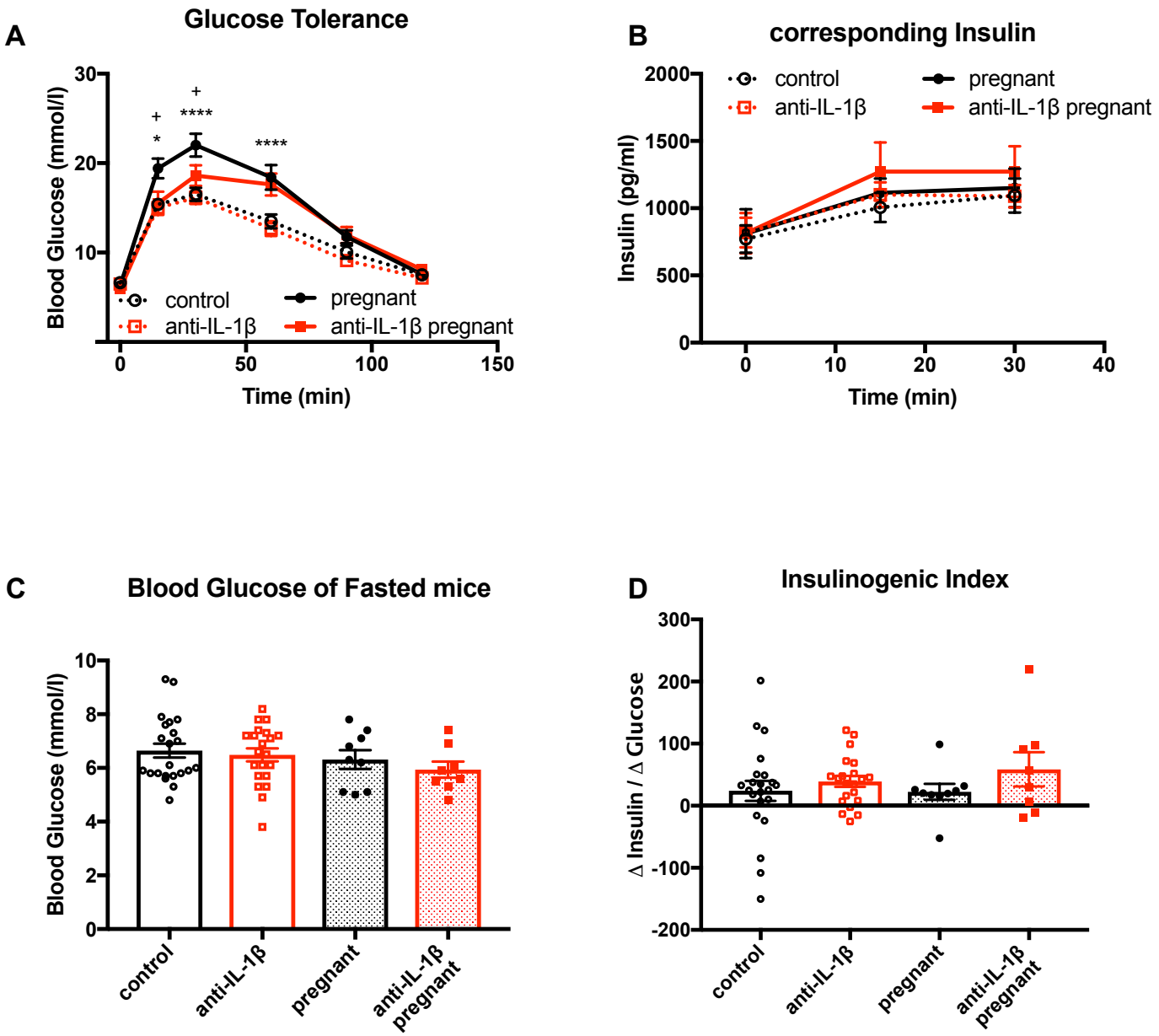


Figure 7

A-D: GDM model with 19-21 weeks old,pregnant and not pregnant mice with or without anti-IL-1β treatment, fed HFD for 3-6 weeks before mating and during pregnancy: **A**: Glucose tolerance, **B**: Corresponding insulin, **C**: Blood glucose in fasted mice, **D**: Insulinogenic index; control n=22, anti-IL-1β: n=21, pregnant n=9, pregnant anti-IL1β: n=7; all data as mean ± SEM, statistics in A and B: 2way ANOVA for repeated measurements with Holm-Sidak multiple comparisons; *= control vs. pregnant, += pregnant vs. anti-IL-1β pregnant.

Figure 8

Healthy Pregnancy Model

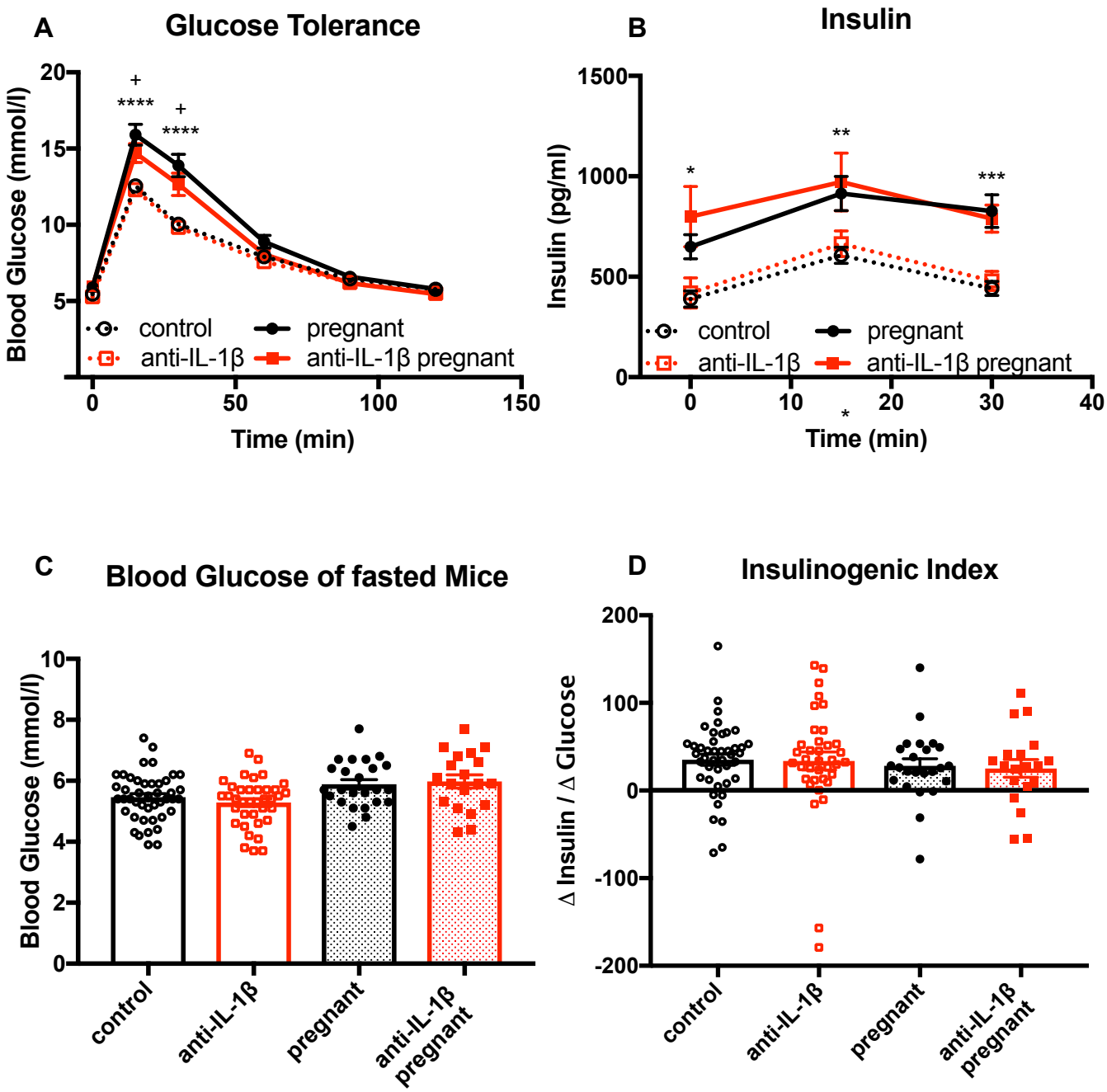


Figure 8

A-D: Healthy pregnancy model with 15-18 weeks old, chow fed pregant and not pregnant mice treated anti-IL-1β or vehicle: **A**: Glucose tolerance, **B**: Corresponding insulin, **C**: Blood glucose in fasted mice, **D**: Insulinogenic index; control n=44, anti-IL-1β: n=37, pregnant n=24, pregnant anti-IL1β: n=19; all data as mean ± SEM, statistics in A and B: 2way ANOVA for repeated measurements with Holm-Sidak multiple comparisons. *= control vs. pregnant, += pregnant vs. anti-IL-1β pregnant.

To test if the anti-IL-1β-induced improvement of the glucose tolerance only occurs in GDM or also in healthy pregnancies, we antagonized IL-1β in 15-18 weeks old healthy pregnant mice. There was a very small, but significant improvement of the glucose tolerance of pregnant mice (Fig. 8A). Plasma insulin, fasting blood glucose and insulinogenic index were unaffected by anti-IL-1β treatment (Fig. 8B-D).

To support the results obtained with anti-IL-1β treatment in the healthy pregnancy model, we performed glucose tolerance tests in constitutive, whole body IL-1β knock out mice (IL-1β KO mice) and their littermate controls (Fig. 9). The mice were 15-19 weeks old and fed a chow diet.

Pregnant control mice had a mild impairment of glucose tolerance (Fig. 9A) and their plasma insulin levels tended to be increased compared to non-pregnant controls (Fig. 9B). The insulinogenic index of pregnant wild type mice tended to be lower than that of non-pregnant controls (Fig. 9D).

The glucose tolerance of pregnant IL-1β KO mice was less impaired, with no significant difference compared to non-pregnant IL-1β KO mice or pregnant control mice. Interestingly, the plasma insulin of pregnant IL-1β KO mice was increased compared to all other groups, basally as well as after the glucose injection. The insulinogenic index of pregnant IL-1β KO mice tended to be better than that of pregnant wild type mice, and that of non-pregnant IL-1β KO mice. The fasting blood glucose levels of all groups were similar (Fig. 9C).

Figure 9: IL-1β KO mice

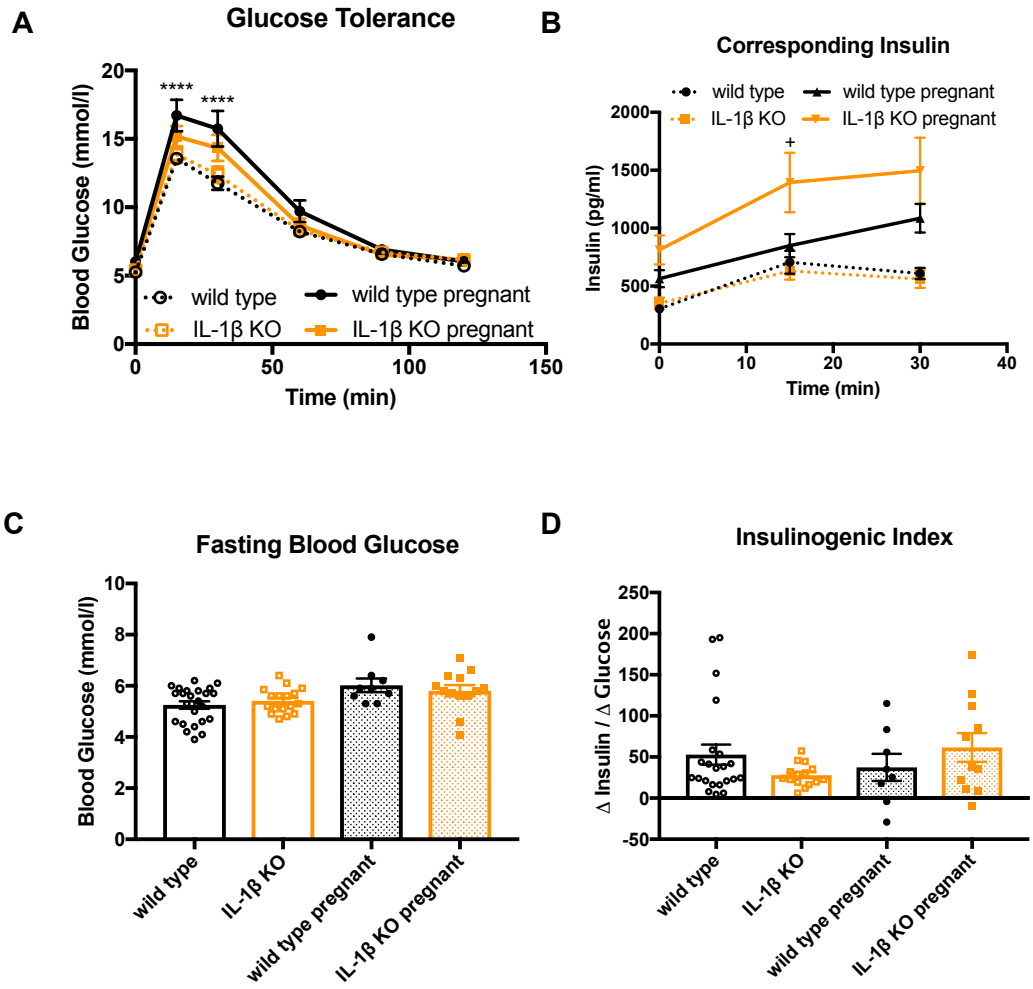


Figure 9:
A-D: Pregnant and not pregnant 15-19 weeks old, chow-fed IL1β KO mice and littermate controls; wild type n=24; IL-1β KO n=17; wild type pregnant n=9; IL-1β KO pregnant n=13; data of 6 cohorts. **A**: Glucose tolerance; **B**: Corresponding plasma insulin; **C**: Fasting blood glucose; **D**: Insulinogenic index; all data as mean ± SEM; statistics: 2-way ANOVA for repeated measurements and Holm-Sidak multiple comparisons; * = wild type vs wild type pregnant; + = wild type pregnant vs. IL-1β KO pregnant

β-Cell area and islet gene expression of proliferation markers

To investigate if anti-IL-1β has an influence on the β-cell mass expansion during healthy pregnancy, we measured the β-cell area on 6-13 serial histological sections per pancreas of mice of the healthy pregnancy model (Fig. 10 and 11). The ratio of β-cell area and total pancreas area serves as a surrogate marker for the β-cell mass.

There was a trend for increased β-cell area, average islet size and number of islets with pregnancy, where the increase of the islet number is the most

Figure 10:

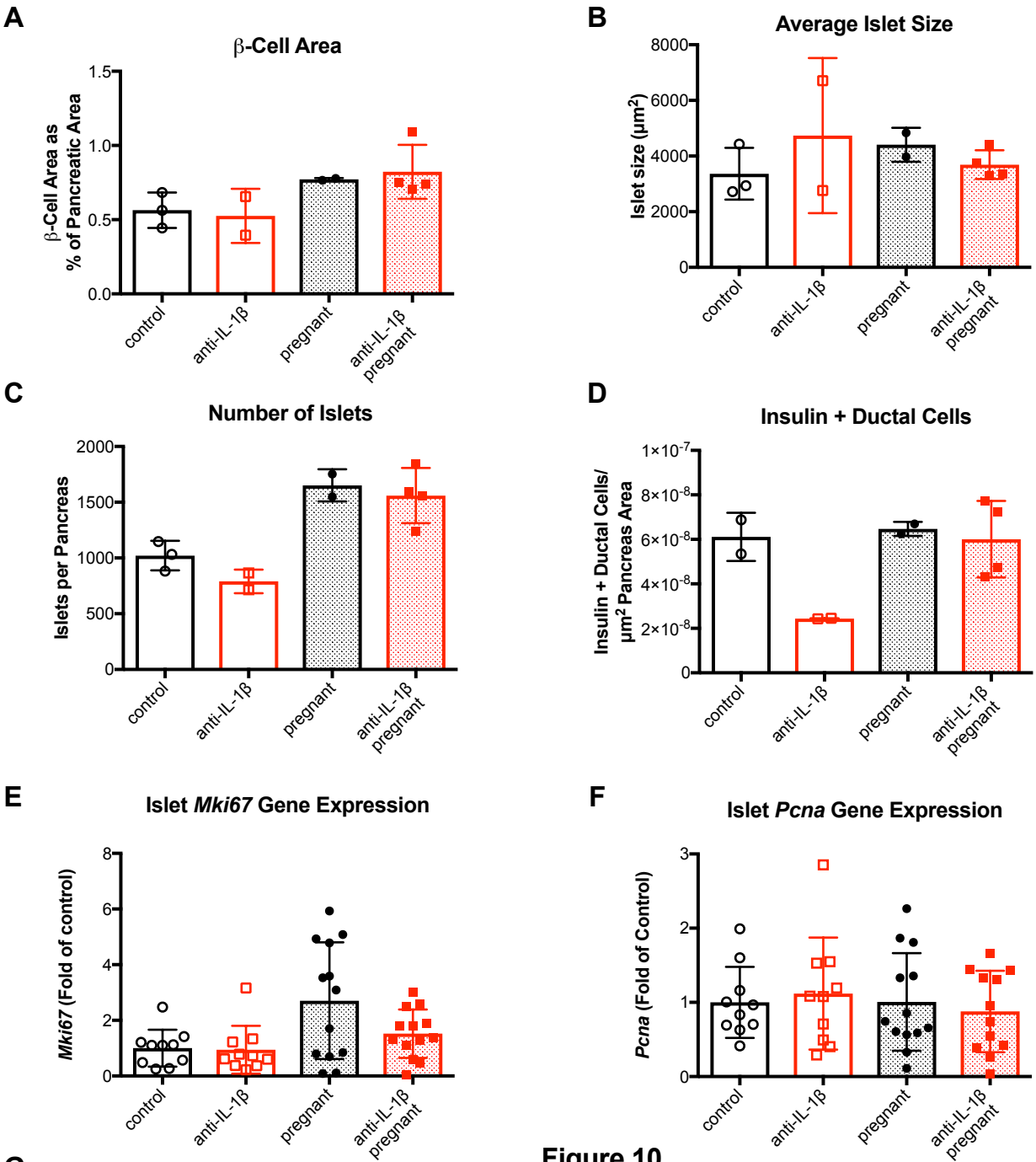


Figure 10

A-D: Quantification of histological data of pancreatic islets in healthy pregnant mice. Each dot represents the average of 6-13 serial sections thoughout the pancreas of one mouse in 100µm distance; control n=3 mice, 31 sections; anti-IL-1β n=2 mice, 19 sections; pregnant n=2 mice, 24 sections; anti-IL-1β pregnant n=4 mice, 45 sections; **E-G**: Islet gene expression of proliferation markers in healthy pregnant mice, **E** and **F:** Control n=10, anti-IL-1β n=10, pregnant n=13, anti-IL-1β pregnant n=13, data from 3 cohorts; **G**: Control n=6, anti-IL-1β n=6, pregnant n=9, anti-IL-1β pregnant n=9, data from 2 cohorts; all data as mean ± SEM

Figure 11

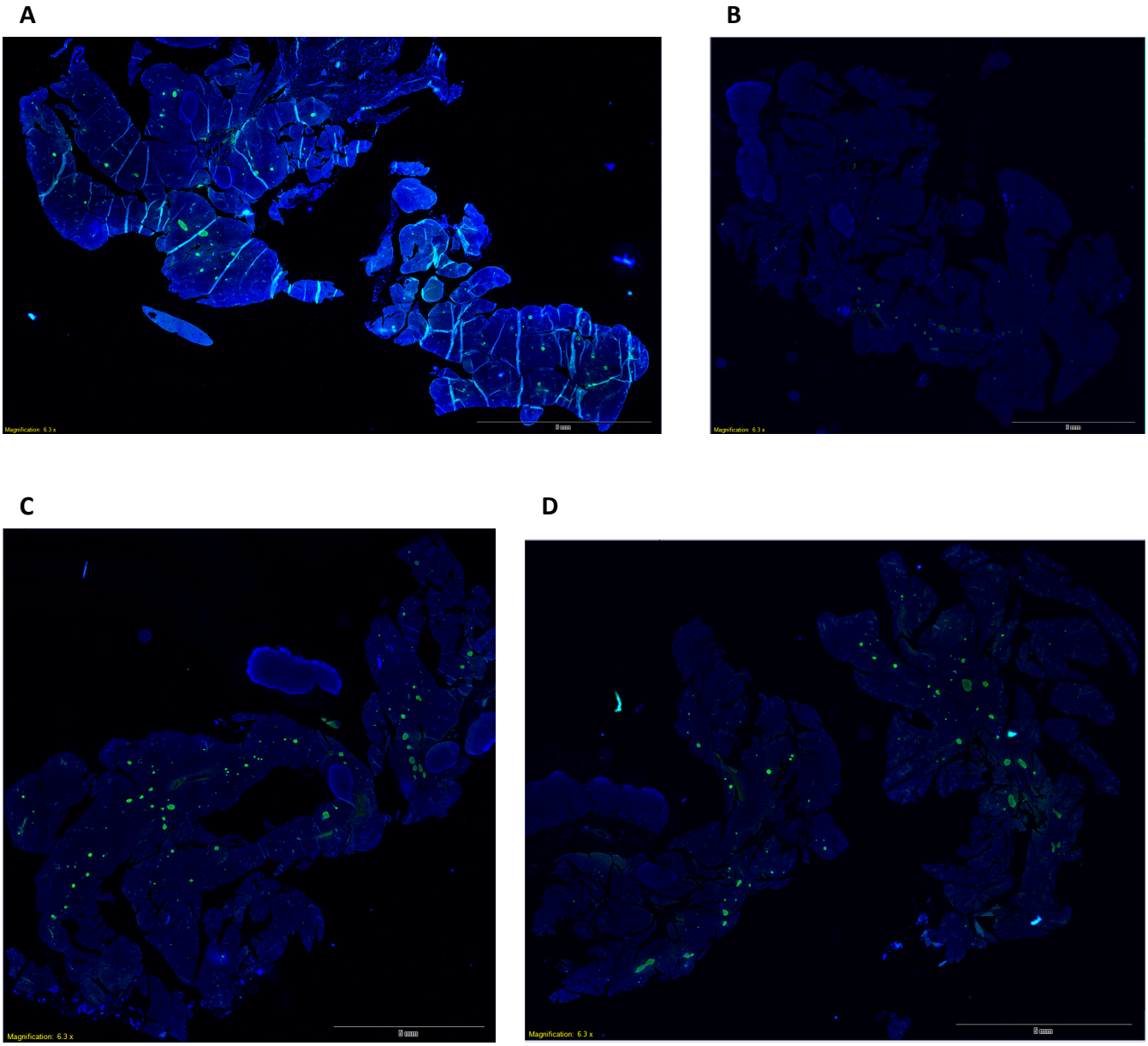


Figure 11
Representative pictures of sections through the middle of the pancreas of chow fed, pregnant and not pregnant mice treated with anti-IL-1β or vehicle: **A**: Control, **B**: Anti-IL-1β **C**: Pregnant **D**: Anti-IL-1β; blue: nuclei stained with DAPI, green: insulin stained with FITC

pronounced. The number of insulin-producing ductal cells per μm^2 pancreas area was similar in pregnant mice and controls (Fig. 10).

Anti-IL-1β didn't influence β-cell area, average islet size or number of islets. But there is a clear trend for less insulin-producing ductal cells in non-pregnant anti-IL-1β-treated mice.

The histological data were complemented by the measurement of the gene expression of the proliferation markers *Mki67*, *Pcna* and *Ccna2* in isolated islets of 12-22 weeks old chow-fed mice (Fig 10). There was no difference in the gene expression of *Pcna* and *Ccna2*, but there was a remarkable trend for increased *Mki67* expression in the islets of pregnant mice, which was reduced with anti-IL-1β treatment.

Insulin tolerance of healthy pregnant mice

To test if IL-1β has an influence on insulin tolerance in pregnant mice and to further characterize the glucose metabolism of pregnant mice we performed insulin tolerance tests in the healthy pregnancy model (Fig. 12). In these tests the blood glucose of mice was measured prior to and 15, 30, 60, 90 and 120 minutes after the subcutaneous injection of an insulin bolus.

We didn't detect a difference in insulin tolerance in pregnant mice compared to non-pregnant controls. Anti-IL-1β neither had an effect on insulin tolerance of pregnant mice nor on that of non-pregnant control mice.

Figure 12: Insulin Tolerance

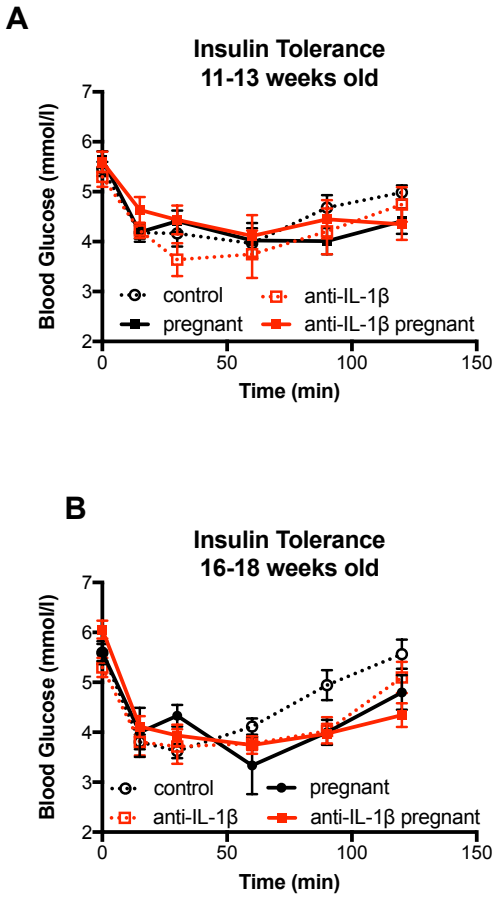


Figure 12
A: Insulin tolerance of 11-13 weeks old chow fed mice; control n=23; anti- IL-1β n=14; pregnant n=9; anti-IL-1β pregnant n=8; data of 2 cohorts. **B**: Insulin tolerance of 16-18 weeks old chow fed mice; control n=14; anti-IL-1β n=7; pregnant n=3; anti-IL-1β pregnant n=7; data of 2 cohorts.
All values are presented as mean ± SEM; statistics: 2-way ANOVA for repeated measurements and Holm-Sidak multiple comparisons.

Organ weight and fetal weight in pregnant mice with anti-IL-1β

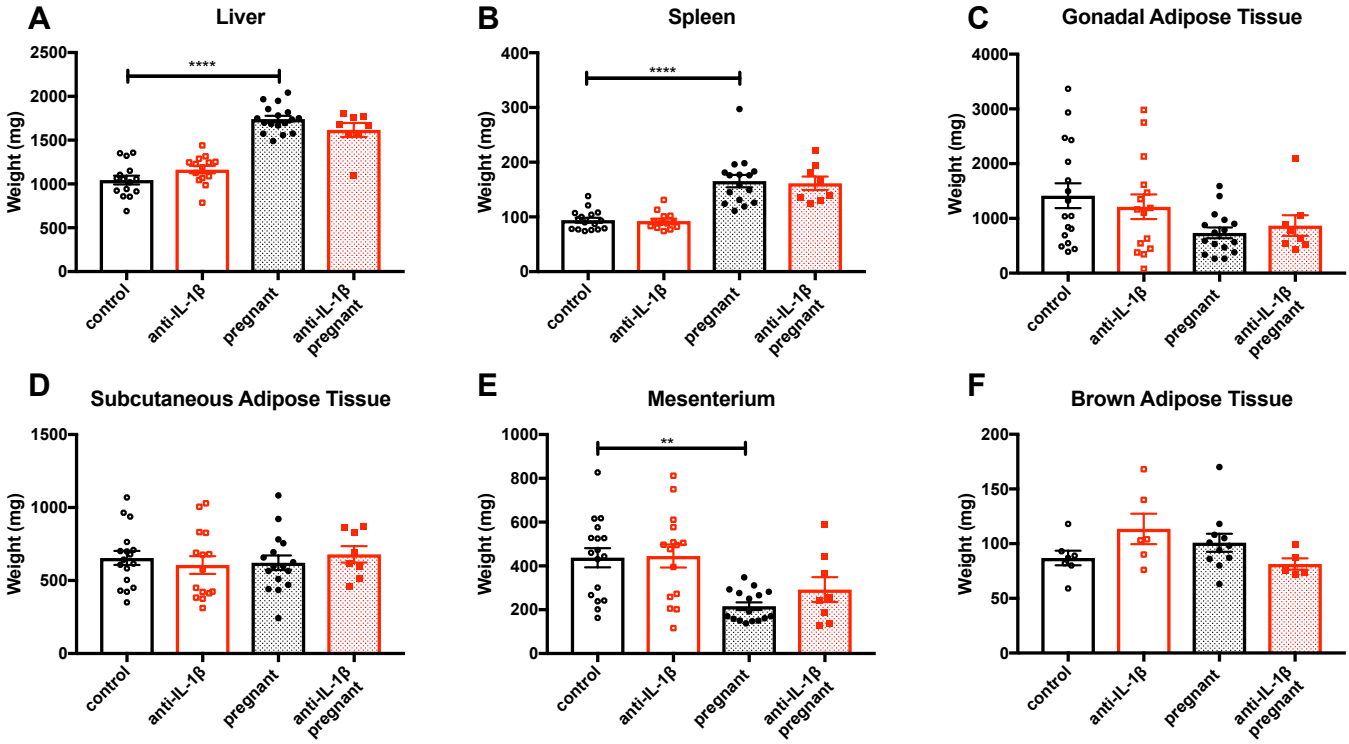
At sacrifice on day 14.5 of pregnancy, the liver, spleen and adipose tissue pads of the dams were weighed (Fig. 13).

Pregnant GDM mice had enlarged livers and spleens, but the weight of the adipose tissue was similar to that of the high-fat diet-fed controls. Anti-IL-1β treatment didn't influence the weight of the organs and adipose tissue pads, although there was a trend for a slightly reduced liver weight in the pregnant anti-IL-1β-treated animals.

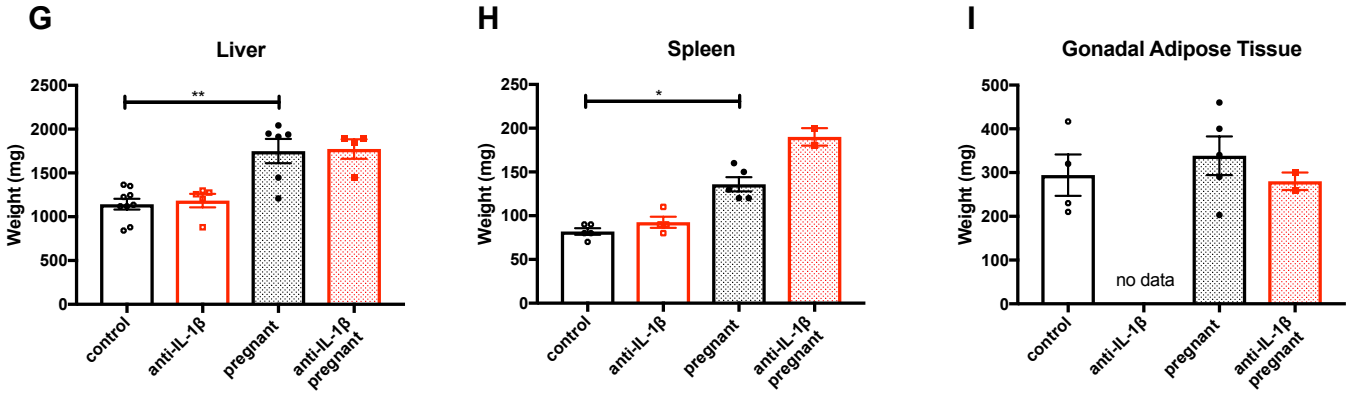
Less data was acquired for healthy pregnant C57BL6/N and healthy pregnant 15-20 weeks old IL-1β KO animals. We measured the weight of the liver, spleen and gonadal adipose tissue in the wild type strain, and the weight of the liver in

Figure 13: Organ Weight

GDM-Model



Healthy Pregnancy Model



Healthy pregnant IL-1β KO Mice

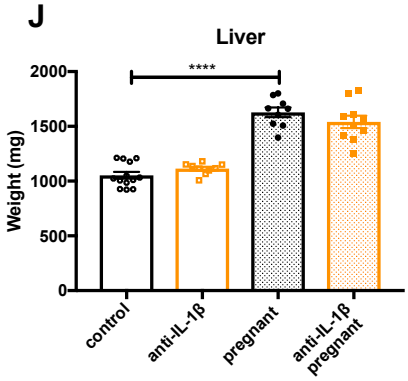
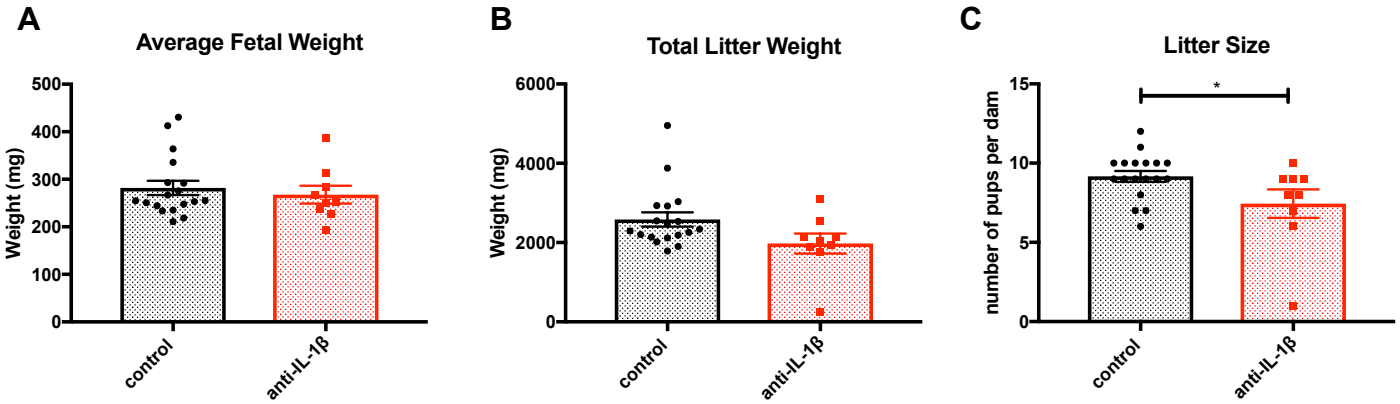


Figure 13

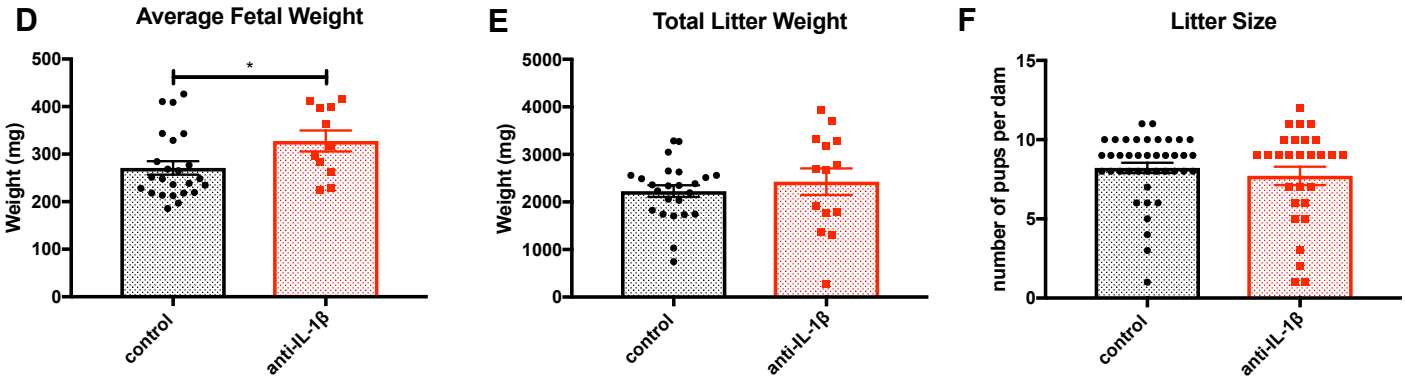
A-F: The weight of different organs and fad pads of GDM mice; **G-I:** The weight of liver, spleen and gonadal fat pads of healthy pregnant mice; **J**: The weight of the liver of healthy IL-1β KO mice, age of mice 15-19 weeks; Each dot represents the weight of the organ/ fat pads of one mouse; all data as mean ± SEM; statistics: Kruskal-Wallis test with Dunn's multiple comparisons

Figure 14: Fetal weight

GDM Model



Healthy Pregnancy model



Healthy Pregnant IL-1β KO Model

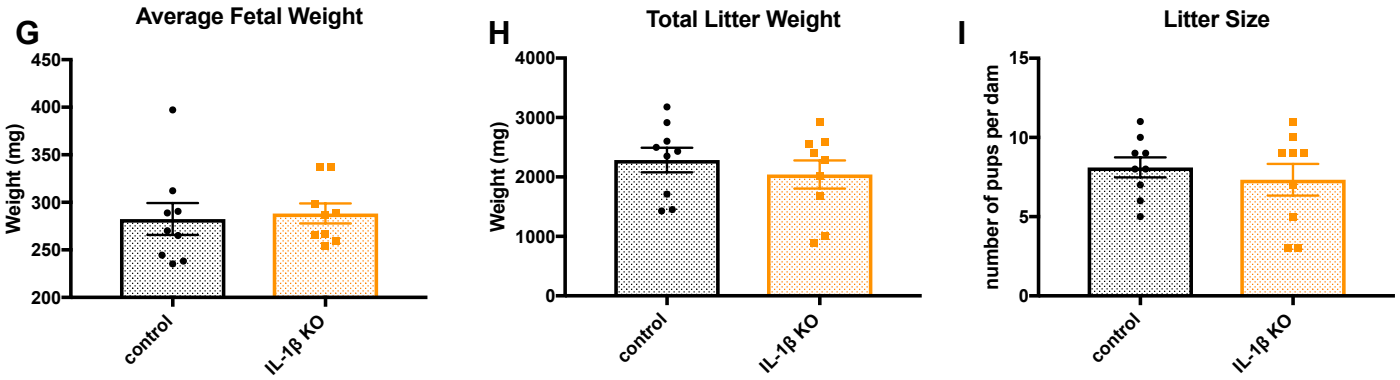


Figure 14

A, B and **C**: Fetal weight in GDM model: control n=16, anti-IL-1β n=7; **D**, **E** and **F**: Fetal weight in 15-18 weeks old healthy pregnant mice: control n=24, anti-IL-1β n=14; **G**, **H** and **I**: Fetal weight in 15-22 weeks old, healthy pregnant IL-1β KO mice: control n=9, IL-1β KO n=9; **A**, **D** and **G**: Average fetal weight; **B**, **E** and **H**: Total litter weight; **C**, **F** and **I**: Litter size

the IL-1β KO strain. The livers and spleens of healthy pregnant mice were enlarged compared to controls, but not the gonadal adipose tissue. Anti-IL-1β treatment and genetic IL-1β deficiency didn't affect the organ weight.

In GDM animals the average fetal weight per dam was independent of the anti-IL-1β treatment (Fig. 14). The litter size in the anti-IL-1β-treated mice was lower in our sample, consequently the total litter weight tended to be lower in the anti-IL-1β-treated mice.
In healthy pregnant mice, the average fetal weight in anti-IL-1β-treated mice was higher than in the controls. The higher average fetal weight didn't translate to a higher total litter weight, despite the same litter size. The average fetal weight, the total litter weight and litter size in IL-1β KO mice was not dependent of the genotype.

Serum steroid hormones

The serum concentration of several steroid hormones was measured in healthy pregnant mice (Fig. 15-17) of one cohort. Serum was sampled at sacrifice, between 8h and 10h in the morning. The panel comprised corticosterone, 11-dehydrocorticosterone, progesterone, 17α-hydroprogesterone, aldosterone, 11-deoxycorticosterone, androstendione and testosterone.
All steroid hormones were increased in pregnant mice (Fig. 15). Interestingly, there was a trend for a reduced serum concentration for every hormone in anti-IL-1β-treated pregnant mice. The difference of the hormone concentrations between non-pregnant anti-IL-1β-treated and pregnant anti-IL-1β-treated mice were not significant for any hormone.
The trend for a reduced serum concentration in anti-IL-1β-treated pregnant mice was most pronounced in 11-deoxycorticosterone.

When the serum concentrations of the steroid hormones are plotted to the litter size, linear regression shows that for some hormones, the litter size and the serum hormone concentrations correlate (Fig. 16).

Figure 15: Serum Steroid Hormones

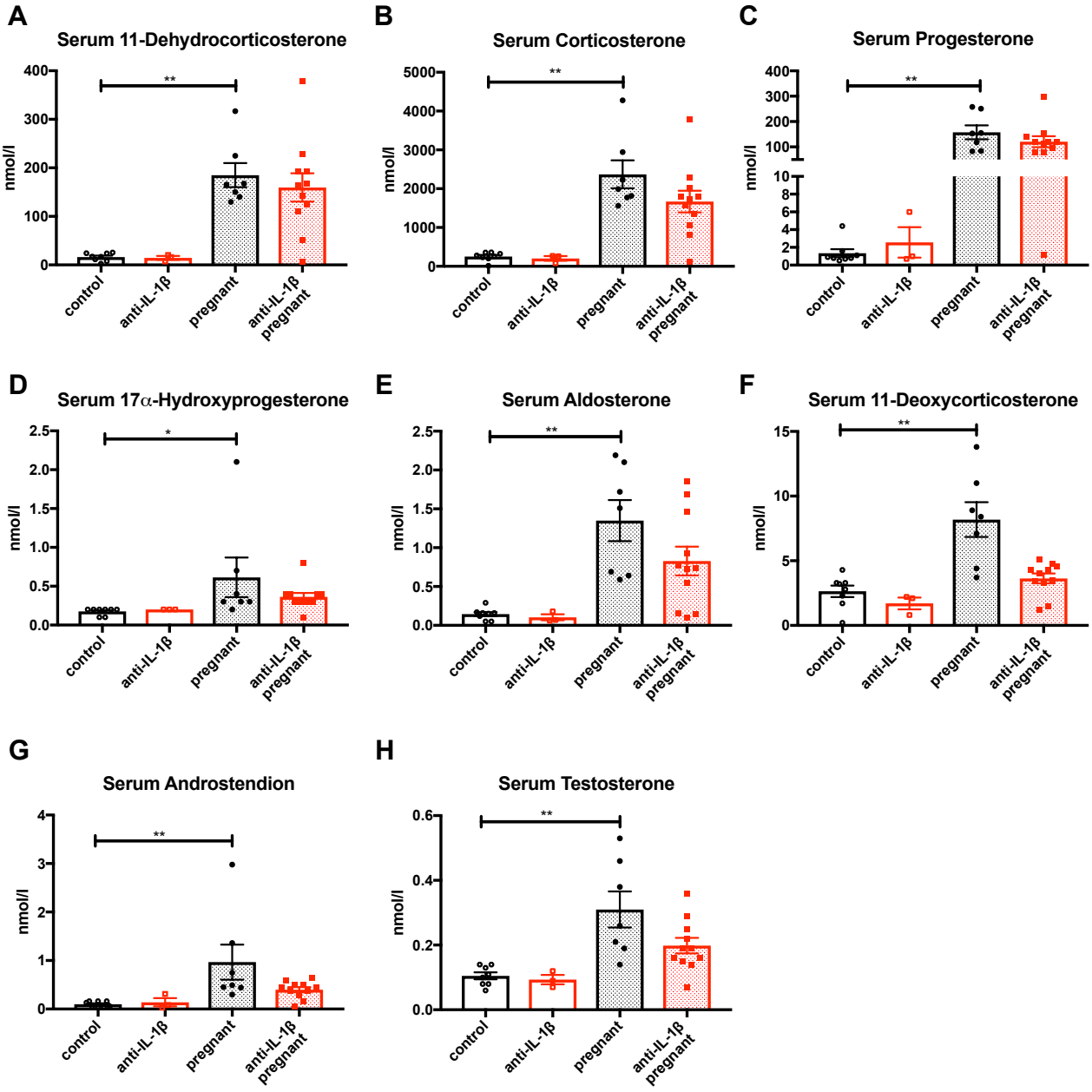


Figure 15

Serum steroid hormon concentrations during pregnancy with and without IL-1β antagonization: mineralocorticoids: **A**: 11-dehydrocorticosteone, **E**: Aldosterone, **F**: 11-deoxycorticosterone; glucocorticoid: **B**: Corticosterone; gestagens: **C**: Progesterone, **D**: 17α-hydroxyprogesterone; androgens: **G**: Androstendion and **H**: Testosterone; data of 1 cohort, chow fed, 12-16 weeks old; control n=8, anti-IL-1β n=3, pregnant n=7, anti-IL-1β pregnant n=11; all data as mean ± SEM, statistics: Kruskal-Wallis test with Dunn's multiple comparisons.

Figure 16

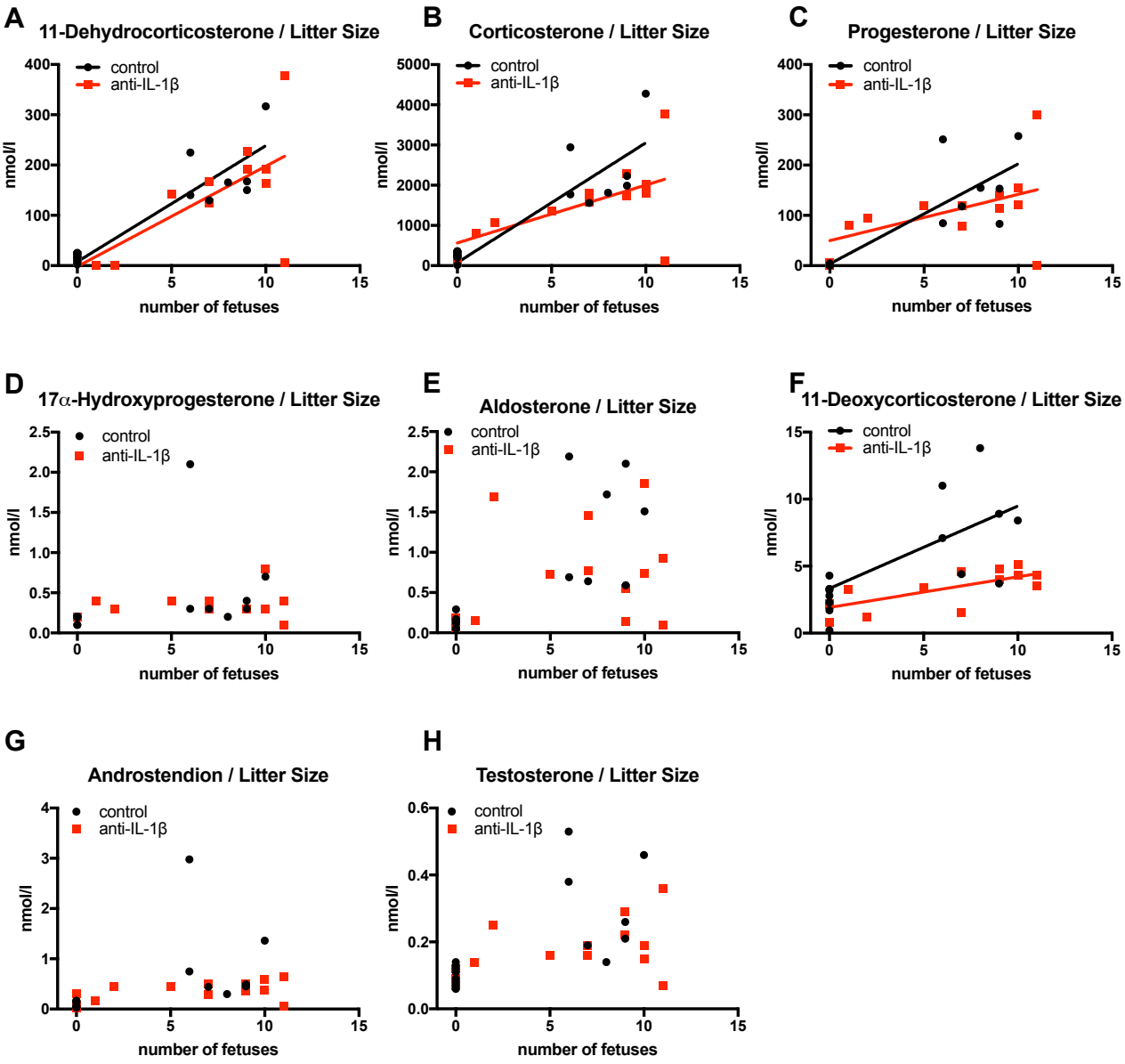


Figure 16

Serum steroid hormon concentrations on y-axis in relation to littersize on x-axis, with and without IL-1β antagonization: mineralocorticoids: **A**: 11-dehydrocorticosteone: control R^2=0.7213, p=0.0324, **E**: Aldosterone: control R^2=0.6558, p=0.0508 , **F**: 11-deoxycorticosterone: control R^2=0.3088, p=0.2522; glucocorticoid: **B**: Corticosterone control R^2=0.6547, p=0.0512; gestagens: **C**: Progesterone: control R^2=0.7162, p=0.0336, **D**: 17α-hydroxyprogesterone: control R^2=0.04819, p=0.676; androgens: **G**: Androstendion: control R^2=0.1194, p=0.5023 **H**: Testosterone: control R^2=0.3274, p=0.2097; data of 1 cohort, chow fed, 12-16 weeks old; control n=8, anti-IL-1β n=3, pregnant n=7, anti-IL-1β pregnant n=11; all data as mean ± SEM, statistics: linear regression analysis

Steroid hormones are generated from other steroid hormones [104]. A comparison of the ratio of a hormone and its precursor between treatment groups indicates if the enzyme that does the transformation is regulated by the treatment. Therefore, we calculated the ratios of the steroid hormones that we measured (Fig. 17).

The ratio of aldosterone to its precursors corticosterone and 11-deoxycorticosterone was neither altered by pregnancy nor by anti-IL-1β treatment. The ratio of corticosterone and 11-dehydrocorticosterone was also similar in every condition. All calculated ratios of progesterone were changed by pregnancy.

Furthermore, there was a trend for a regulation by anti-IL-1β of the ratios of progesterone and aldosterone, corticosterone and 11-deoxycorticosterone respectively, always in favor of relatively more progesterone in the anti-IL-1β-treated conditions, independent of pregnancy.

Figure 17

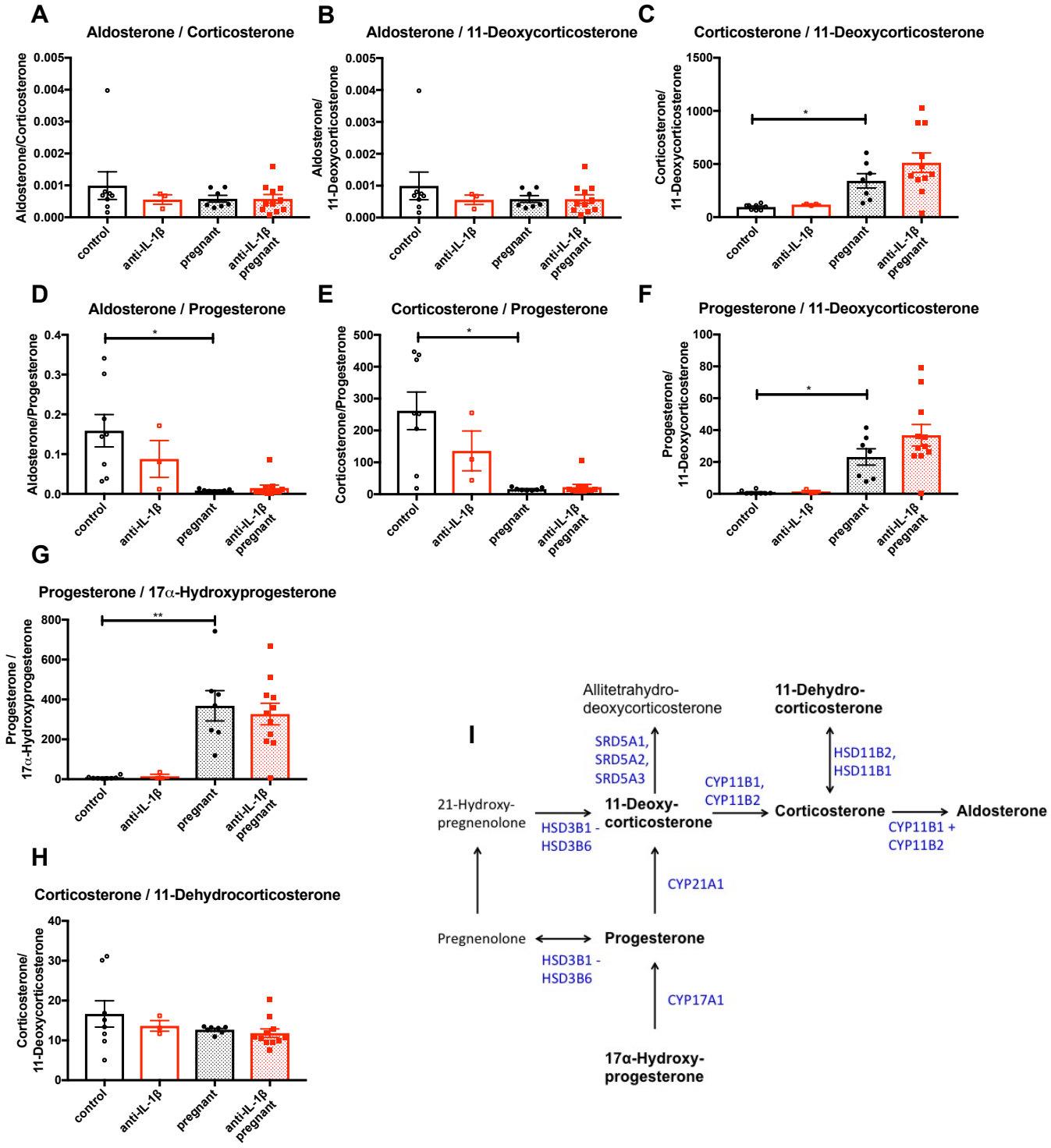


Figure 17

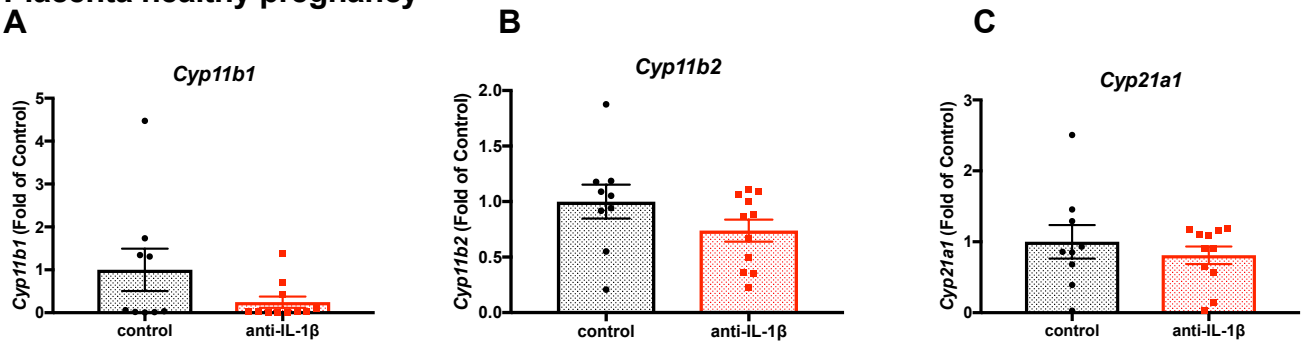
Ratios of serum steroid hormon concentrations during pregnancy, with and without IL-1β antagonization: **A**: Aldosterone/ corticosterone, **B**: Aldosterone /11-deoxycorticosterone, **C**: Corticosterone/ 11-deoxycorticosterone, **D**: Aldosterone/ prgogesterone, **E**: Corticosterone/ progesterone, **F**: Progesterone/ 11-deoxycorticosterone, **G**: Progesterone/ 17α-hydroxyprogesterone, **H**: Corticosterone/ 11-dehydrocorticosterone; data of 1 cohort, chow-fed, 12-16 weeks old; control n=8, anti-IL-1β n=3, pregnant n=7, anti-IL-1β pregnant n=11; all data as mean ± SEM, statistics: Kruskal-Wallis test with Dunn's multiple comparisons; **I**: Map of steroid hormone synthesis adapted from the KEGG library [104]: bold: measured hormones, blue: enzymes katalysing the reaction indicated by the arrow.

Also, the ratio of corticosterone and 11-deoyxcorticosterone is influenced by pregnancy, and there is a trend for relatively more corticosterone relative to 11-deoxycorticosterone in pregnant anti-IL-1β-treated mice.

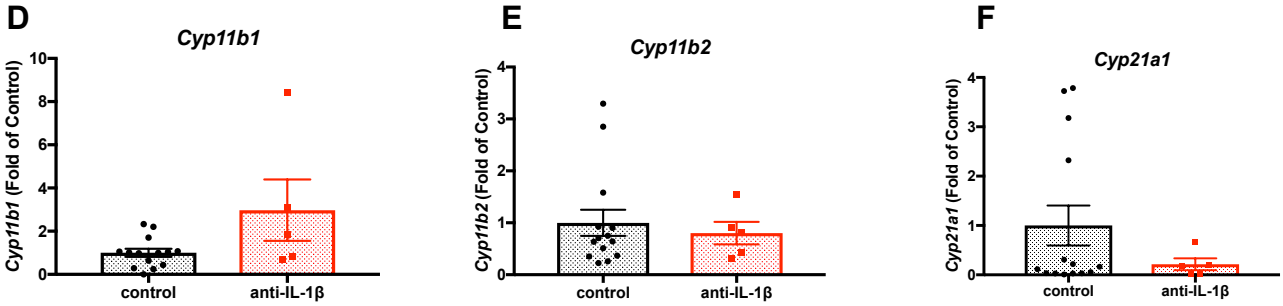
To investigate if the trend for altered enzyme activity in anti-IL-1β-treated mice is due to altered gene expression of the respective enzymes we measured the gene expression of *Cyp21a1* and *Cyp11b1* and *Cyp11b2* in the placenta, the adrenals and the ovaries of healthy pregnant dams and dams with GDM (Fig. 18). There was no significant difference in the gene expression between controls and anti-IL-1β-treated mice. With anti-IL-1β treatment, there were trends for reduced *Cyp11b1* expression in the placenta of healthy pregnant mice and the

Figure 18: Placenta, Adrenal and Ovary gene expression

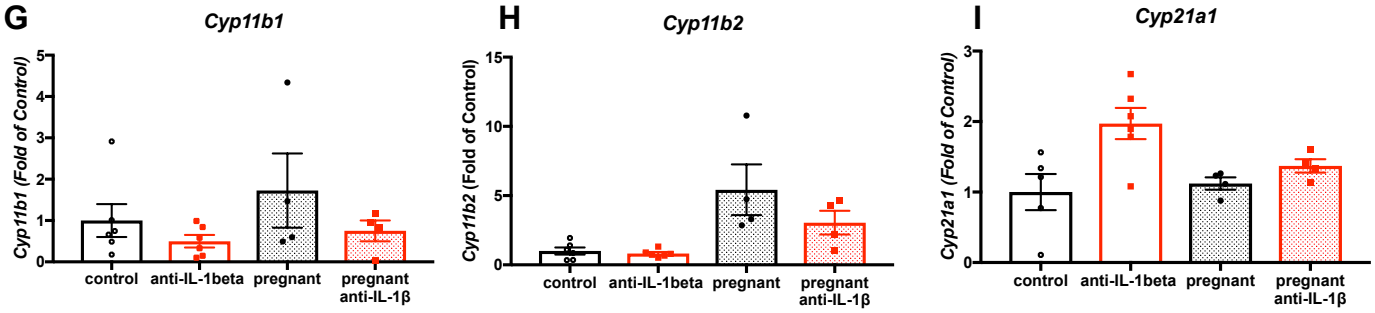
Placenta healthy pregnancy



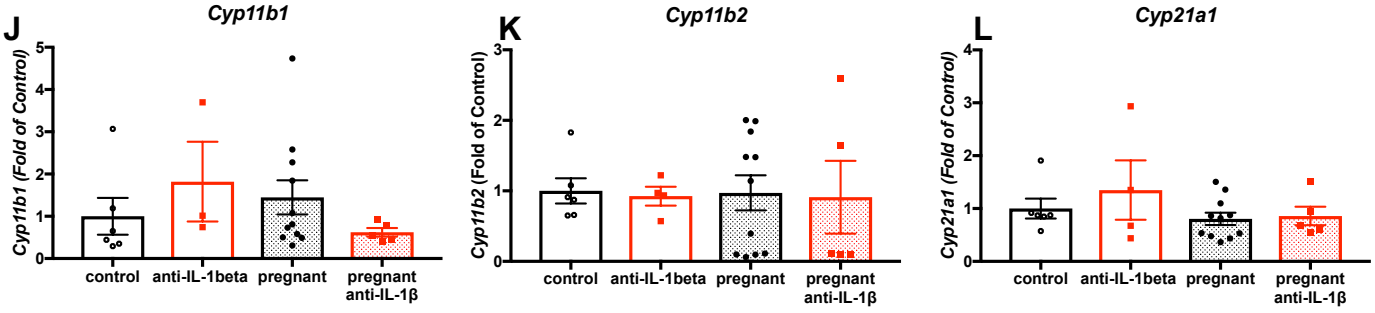
Placenta GDM



Ovaries healthy pregnancy



Ovaries GDM



Adrenals GDM

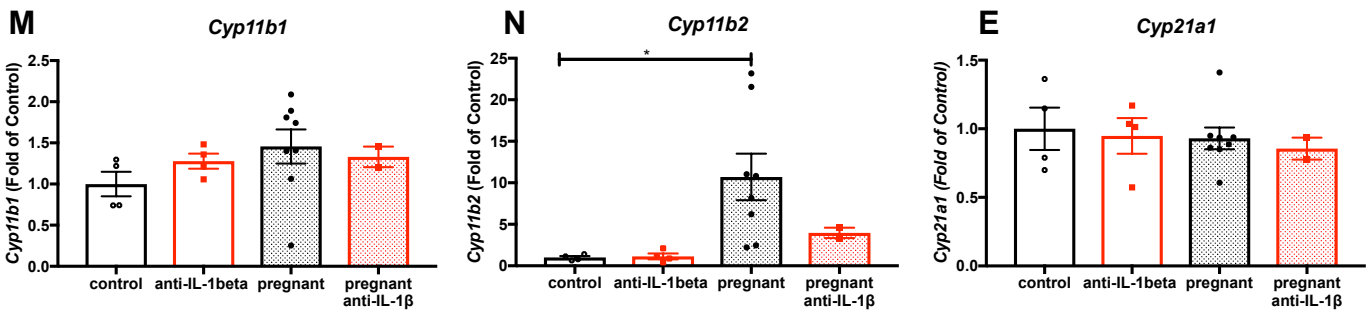


Figure 18
Gene expression of *Cyp11b1, Cyp11b2* and *Cyp21a1* in placenta, ovaries and adrenals expressed as fold of control relative to the reference genes *Actinb* and *Gapdh.* **A**-**C**: Placental gene expression in healthy pregnant mice: control n=9, anti-IL-1β n=11; **D**-**F**: Placental gene expression in GDM model: control n=14, anti-IL-1β n=5; **G**-**I**: Ovarian gene expression in healthy pregnant mice: control: n=6, anti-IL-1β: n=6, control pregnant: n=4, anti-IL-1β pregnant: n=4; **J**-**L**: Ovarian gene expression in GDM mice: control: n=6, anti-IL-1β: n=4, control pregnant: n=11, anti-IL-1β pregnant: n=5, **M**-**O**: Adrenal gene expression in GDM mice: control: n=4, anti-IL-1β: n=4, control pregnant: n=8, anti-IL-1β pregnant: n=2, all data as mean ± SEM.

ovaries of healthy pregnant and GDM mice, reduced *Cyp11b2* expression in the ovaries of healthy pregnant mice and the adrenals of GDM mice and reduced *Cyp21a1* expression in placentas of GDM mice.

LysMCreIL-1β KO model

To test if the IL-1β that influenced glucose tolerance of pregnant mice derives from cells of the myeloid lineage, we mated high-fat diet-fed LysMCre-IL-1β KO mice and their littermate controls. The data of this experiment derive from three glucose tolerance tests with one mouse cohort, which was mated repeatedly. Therefore, the age of the mice ranged between 11 and 18 weeks (Fig. 19). The glucose tolerance of pregnant wild type mice was impaired compared that of wild type controls, and they had higher plasma insulin levels. LysMCre-IL-1β KO mice tended to have an improved glucose tolerance compared to wild type controls, both in pregnant and in non-pregnant mice. In non-pregnant controls, the improvement of glucose tolerance of LysMCre-IL-1β KO mice was significant. The plasma insulin of LysMCre-IL-1β KO mice tended to be lower, both in pregnant and in non-pregnant mice. Fasting blood glucose and the insulinogenic index were not different.

Figure 19: Glucose tolerance of LysMCre-IL1β KO mice with GDM

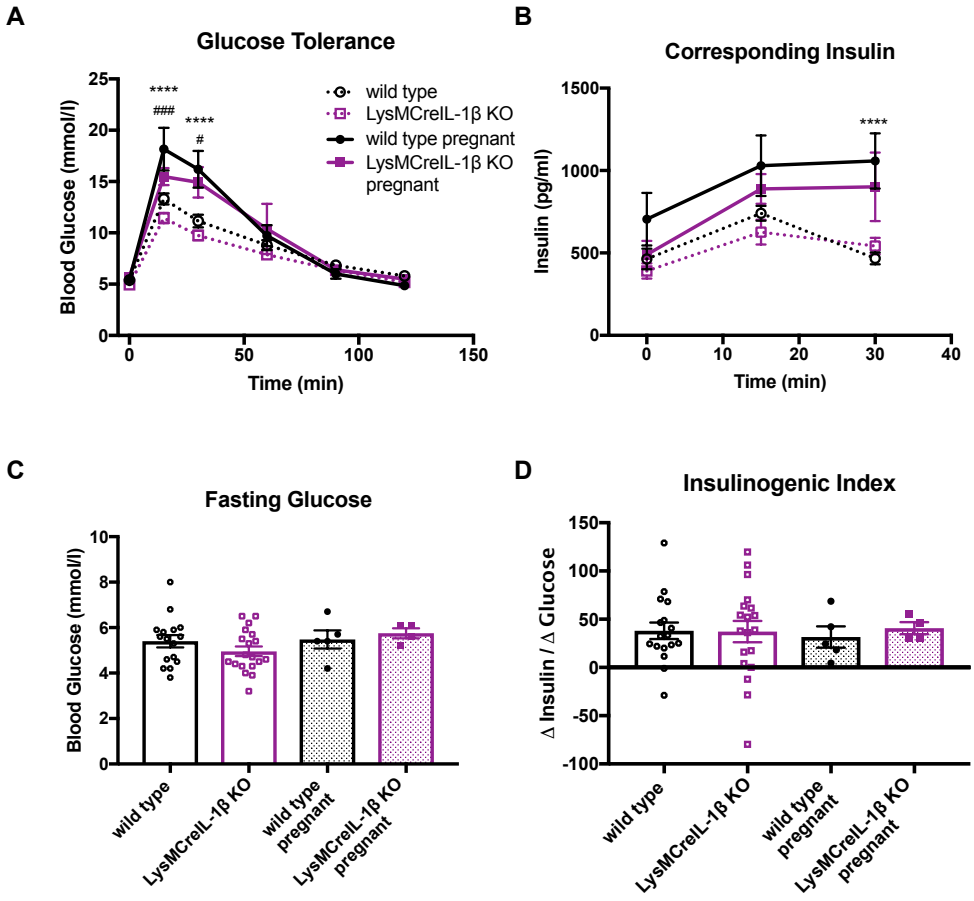


Figure 19

A-**D**: 11-18 weeks old, HFD fed, pregnant and not pregnant LysMCre-IL1β KO mice and littermate controls:

A: Glucose tolerance; **B**: Corresponding Insulin; **C**: Fasting Glucose; **D**: Insulinogenic index, wild type: n=18, LysMCre-IL-1β KO: n=20, wild type pregnant: n=5, LysMCre-IL1β KO pregnant: n=4; data as mean ± SEM, statistics: A, B: 2way ANOVA with Holm-Sidak multiple comparisons; * = wild type vs. wild type pregnant, # = wild type vs. LysMCre-IL1β KO

Discussion

A mouse model of gestational diabetes

On day 13.5 of normal, healthy mouse pregnancies, glucose tolerance, tested by subcutaneous glucose tolerance tests, was impaired and insulin secretion was increased in 10-13 weeks old as well as in 15-18 weeks old mice. These results are in line with i.p. glucose-tolerance tests performed in pregnant mice on day 16.5 [25, 105].

Insulin secretion of pregnant mice was increased basally as well as during the tests, while glucose tolerance was impaired. This indicates that the glucose-stimulated insulin secretion of pregnant mice was not increased compared to that of control mice, which is also illustrated by the insulinogenic index. The impaired glucose tolerance, concurrent with the increased insulin secretion is indicative for the expected insulin resistance during pregnancy [25].

The age of the mice didn't change the glucose phenotype. We speculate that 15-18 weeks are not old enough to model high maternal age-induced GDM.

High-fat diet feeding led to pronounced glucose-tolerance impairment in pregnant mice compared to non-pregnant controls. This occurred when mice received the high-fat diet either only during pregnancy, or already before the onset of pregnancy. The peak plasma glucose concentration in pregnant animals was at 30 minutes after glucose injection instead of after 15 minutes in chow-fed pregnant and control mice. Late blood glucose peaks during glucose-tolerance tests are commonly observed in insulin resistant animals [106-109].

Pregnant mice, which received high-fat diet feeding only during pregnancy, had highly increased insulin secretions, which might partly compensate for the presumably increased insulin resistance. In contrast, pregnant mice, which received high-fat diet already before pregnancy were not able to mount a sufficiently high insulin response. Their plasma insulin was, despite impaired glucose tolerance, not higher than that of the non-pregnant controls, suggesting β-cell failure. The insulinogenic index of pregnant mice and control mice was equal in both groups, but it was higher in mice, which received high-fat diet only for a

short time during pregnancy than in those which received high-fat diet for a longer period, which also reflects the impaired insulin response in the latter.

Based on our data, together with the known risk for obese women to develop GDM, we conclude that high-fat diet feeding with onset before pregnancy in relatively old mice is a good model to study GDM.
When the data of all four models tested are taken into account, the glucose-tolerance impairment during pregnancy correlated with the weight of the mouse before pregnancy. This further supports the model.

However, there are also limitations to the high-fat diet-induced GDM model. The fasting blood glucose of the pregnant mice was not increased compared to that of non-pregnant control mice.
And, more important, the fetal weight of dams fed high-fat diet was not increased compared to that of dams fed normal chow. This might be explained by the slightly increased litter size (the more fetuses there are, the lower is the weight of the average fetus [110]) of our sample, but also to the gestational age. The day on which we sacrificed the animals was at the beginning of the third trimester, which is the important phase of fetal weight gain [111]. In a study performed on day 18.5 of pregnancy, high-fat diet increased the fetal weight in C57BL/6 mice [112]. This discrepancy to our results may also be explained by a different composition of the high-fat diet used. It contained larger proportion of calories coming from carbohydrates than our high-fat diet.

High-fat diet feeding influenced the fertility of the mice: There was a trend for more mice becoming pregnant during timed mating in high-fat diet-fed cohorts compared to chow-fed cohorts. This may stem from altered sex hormone levels as described by Whyte *et al*. [113]. It has been reported that high-fat diet feeding reduces fertility [114], but in many studies also male mice received high-fat diet for which a reduced fertility with high-fat diet has been reported [115]. Other explanations could be the composition of our high-fat diet and the duration of high-fat diet feeding before mating.

In the following sections, the model with 19-20 weeks old mice and high-fat diet feeding before and during pregnancy will be called GDM model, the model with 10-18 weeks old, chow-fed mice will be called healthy-pregnancy model.

We hypothesized, that increased IL-1β contributes to the glucose intolerance during pregnancy and in GDM. Indeed, serum IL-1β was increased in pregnant mice, both in healthy pregnancy and in the short-term high-fat diet model. However, the difference was not detectable when the mice were fasted, which is the case before glucose-tolerance testing. But IL-1β is a highly potent cytokine, of which locally high concentrations can have important effects, without a systemically detectable difference or without being detectable at all [116]. Thus IL-1β might influence glucose tolerance during pregnancy also in fasted mice.
Consequently, we measured *Il1b* gene expression, in organs where we suspected a difference between pregnant and non-pregnant mice: In organs that influence the glucose metabolism such as pancreatic islets and the insulin-target tissues liver, muscle and subcutaneous adipose tissue, in peripheral blood cells and in the uterus with the fetal-maternal interface.
We found higher *Il1b* gene expression in the uterus of pregnant GDM mice, and a trend for higher *Il1b* gene expression in the uterus of healthy pregnant mice. *Il1b* gene expression normalized to the expression of *18s* in the uterus may even underestimate the real increase during pregnancy, as also the *18s* expression, as well as the expression of 10 other reference genes (we show *Actinb* and *Gapdh*) was increased. This underlines the difference of a normal uterus and the modified uterus during pregnancy.
If the concentration of active IL-1β protein is increased in the uterus of pregnant mice, and if this can contribute to increased systemic IL-1β remains to be investigated.

Blocking IL-1β signaling improves glucose tolerance of pregnant mice

The best way to test if IL-1β has an effect on a parameter like glucose tolerance, is to antagonize it and measure the parameter without it [116]. Blocking IL-1β by injecting anti-IL-1β during the second trimester improved glucose tolerance of

pregnant mice of the GDM model and, to a lesser extent, of the healthy pregnancy model. The latter was supported by experiments with IL-1β KO mice, which are constitutively IL-1β-deficient and not only during the second trimester of pregnancy.

The glucose tolerance of the non-pregnant control mice was not improved by IL-1β antagonism or deficiency, suggesting a pregnancy-specific IL-1β effect. The control mice in the GDM model received high-fat diet, thus the pregnancy specificity is in conflict with studies showing that IL-1β antagonism or IL-1β deficiency improves glucose tolerance in high-fat diet-fed mice [106, 107]. These studies examined male mice, which are more susceptible to diet-induced obesity and impaired glucose control than females [117]. This sex difference and the duration and different compositions of the diets used in [106, 107] compared to our high-fat diet might explain the discrepancy.

Since the glucose tolerance of anti-IL-1β-treated and IL-1β-deficient pregnant mice was only partially restored and still impaired compared to that of non-pregnant controls, it is evident that IL-1β cannot be the only trigger for pregnancy-induced glucose intolerance.

Insulin secretion of pregnant mice of the GDM model and the healthy pregnancy model was not significantly improved by anti-IL-1β treatment, but the insulinogenic index of pregnant anti-IL-1β-treated mice of the GDM model tended to be improved compared to that of the not treated mice, pointing to a better insulin response to the glucose stimulus.

The insulin secretion of healthy pregnant IL-1β KO mice was notably higher than that of littermate controls. This may be due to the fact that the IL-1β deficiency in the IL-1β-KO mice was already present before and at the beginning of pregnancy, influencing the capability of the pancreatic islets to adapt to stressors like pregnancy. The mean insulinogenic index in the pregnant IL-1β-KO mice was better, although not statistically significant with our sample size.

In summary, neutralizing IL-1β and genetic IL-1β deficiency improved glucose tolerance in pregnant mice, and this beneficial effect was even more pronounced in GDM. The improvement was specific for pregnancy and didn't occur in high-fat diet-fed control mice, which were glucose intolerant compared to chow-fed

control mice. The effect of IL-1β antagonism might be achieved through better insulin secretion, thus an effect on pancreatic β cells. However, the effect on insulin secretion was mild in the anti-IL-1β-treated mice, suggesting additional effects on either insulin sensitivity of the mother or on glucose transfer to the fetus.

β-Cell mass

Higher insulin secretion during pregnancy is the function of higher β-cell mass [32]. Assuming that anti-IL-1β treatment influences insulin secretion, we may hypothesize that anti-IL-1β treatment increases β cell-mass expansion during pregnancy. Moreover, IL-1β antagonism with XOMA 052, a neutralizing anti-IL-1β antibody, increased β-cell mass in high-fat diet-fed mice through increased β-cell proliferation and decreased β-cell apoptosis [106].

In contrast to this, we didn't observe an increase of β-cell mass (expressed as β-cell area/pancreatic area on histological sections) in healthy pregnant mice treated with anti-IL-1β compared to pregnant control mice.

Ki-67 is a commonly used proliferation marker. It is associated with ribosomal RNA transcription [118]. In islets isolated from pregnant mice during the second trimester (day 10.5 and 14.5), the gene expression of *Mki67*, the gene encoding for Ki-67, is increased, probably reflecting the increased β-cell proliferation [119]. We saw an increased gene expression of *Mki67* in islets of healthy pregnant mice as well. However, in islets isolated from anti-IL-1β-treated pregnant mice the *Mki67* gene expression was unexpectedly reduced to the levels of non-pregnant controls. More studies about β-cell mass and β-cell proliferation in the GDM model and in IL-1β KO mice will be needed to clarify the role of IL-1β in pregnancy-induced β cell-mass expansion.

Insulin tolerance, organ weight and fetal weight

To test if antagonizing IL-1β has an effect on insulin sensitivity during pregnancy we performed insulin-tolerance tests in healthy pregnant mice on day 13.5 of pregnancy. In contrast to studies done on day 16.5 of pregnancy [25, 105], we didn't see pregnancy-induced insulin resistance using insulin-tolerance tests.

Consequently, we cannot conclude on an effect of IL-1β antagonism on insulin sensitivity with this test.

Since insulin regulates cell growth and glycogen and lipid storage, a difference in insulin sensitivity might influence the weight of organs. We measured the weight of various organs and adipose tissue pads in pregnant mice of the GDM model and healthy pregnant mice with and without anti-IL-1β treatment. We saw the expected pregnancy-induced weight increase of the liver [120] and the spleen [121], but there was no pregnancy-induced weight gain of the subcutaneous and the gonadal adipose tissue, which has been reported by Zhang *et al.* [122]. The weight of the gonadal adipose tissue and the mesenterium (containing the visceral adipose tissue) was even decreased in pregnant mice of the GDM model compared to the respective non-pregnant controls. This may be due to the insulin resistance-induced increased lipolysis [123]. IL-1β antagonism didn't reveal effects on organ weights with the sample size analyzed.

The fetus is an important glucose sink in the body of the mother. The improvement of glucose tolerance with IL-1β antagonism could be due to increased glucose clearance by the placental-fetal unit. To approach this, we measured fetal weight. The average fetal weight of healthy pregnant mice was indeed higher in anti-IL-1β-treated mice, but this difference was not large enough to translate into increased total litter weight. In the mice of the GDM model and in IL-1β-KO mice, there was no difference in fetal weight.
More studies will be needed to look at insulin sensitivity and fetal glucose uptake in pregnant mice treated with anti-IL-1β.

Serum steroid hormones

Steroid hormones contribute to pregnancy-induced insulin resistance [28] and might be regulated by IL-1β [65-69]. We therefore measured a panel of serum steroid hormone concentrations in pregnant mice with and without anti-IL-1β treatment. We did this in the healthy-pregnancy model in the morning (8.30-9.30 a.m.).

All hormones measured were increased during pregnancy compared to non-pregnant controls. Interestingly, IL-1β antagonism tended to reduce all steroid hormone concentrations.

The concentrations of corticosterone, progesterone, 11-dehydrocorticosterone and 11-deoxycorticosterone correlated with the litter size, suggesting that these hormones were predominantly produced by the placental-fetal unit. The correlation of maternal serum corticosterone levels and litter size has been described before [124].

However, it has been published that in pregnant mice, progesterone is a product of the ovaries (with one corpus luteum per fetus) [125], where their secretion is stimulated by placental lactogen I and II, and corticosterone a product of the maternal adrenals [126, 127]. Nevertheless, the placenta is able to produce the hormones mentioned above and contributes to their high serum concentrations during pregnancy [127-129].

With anti-IL-1β treatment, the ratio of progesterone to every other hormone was tilted toward more progesterone, also in the non-pregnant control mice. The ratio of 11-deoxycorticosterone to corticosterone was tilted toward corticosterone. The ratios of corticosterone to aldosterone and 11-dihydrocorticosterone were neither influenced by pregnancy, nor by anti-IL-1β treatment. This suggests that IL-1β downregulated the conversion of progesterone to 11-deoxycorticosterone and upregulated the conversion of 11-deoxycorticosterone to corticosterone.

Progesterone and corticosterone have been associated with pregnancy-induced insulin resistance [25, 28], therefore tilting the ratios to relatively more progesterone and corticosterone in anti-IL-1β-treated mice cannot explain an improvement of their glucose tolerance. However, 11-deoxicorticosterone is known to induce gluconeogenesis and reduce insulin sensitivity in rats [130]. It may be that the relative increase in progesterone and corticosterone is rather a relative decrease in 11-deoxycorticosterone. In absolute values all three hormones were reduced in anti-IL-1β-treated animals.

We tested if anti-IL-1β treatment influences the gene expression of the enzymes responsible for the conversions of progesterone to 11-deoxycorticosterone and 11-deoxycorticosterone to corticosterone in the placenta, the maternal adrenals and the maternal ovaries analogue. In pregnant anti-IL-1β-treated mice of the

healthy pregnancy and GDM model, we observed trends for reduced expression of *Cyp11b1* and *Cyp11b2* (encoding for steroid-11β-hydroxylase, which is responsible for the conversion of 11-deoxycorticosterone to corticosterone) in several organs. This does not fit to relatively less 11-deoxycorticosterone in anti-IL-1β-treated mice. We also observed a trend for reduced expression of *Cyp21a1* (encoding for steroid-21-hydroxylase, which is responsible for the conversion of progesterone to 11-deoxycorticosterone) in the placentas of GDM mice, which would fit to relatively less 11-deoxycorticosterone. Collectively the data on *Cyp11b1, Cyp11b2* and *Cyp21a1* gene expression do not indicate that IL-1β regulates steroid hormone synthesis through direct influence on the gene expression of the involved enzymes.

In summary, we observed increased serum steroid hormone concentrations during pregnancy and trends for reduced steroid hormone concentrations in healthy pregnant mice treated with anti-IL-1β. We assume that the placenta either directly or indirectly regulates the production of these steroid hormones, and that the placenta is therefore the place where IL-1β most probably exerts an effect during pregnancy. However, it is unlikely that IL-1β directly regulates the gene expression of the enzymes involved in the steroid synthesis.

More studies are needed to find causal relations between IL-1β, steroid hormone synthesis and glucose metabolism during pregnancy. An important steroid hormone missing in our panel is estradiol, which at high levels induces insulin resistance, but at lower levels preserves insulin sensitivity [57].

In addition, measuring the concentrations of placental lactogen I and II might be of interest, as they are known to regulate progesterone secretion from the ovaries during mouse pregnancy [125]. Furthermore, IL-1 signaling stimulates the release of pituitary prolactin and growth hormone in rats [69]. Prolactin and placental lactogen are closely related hormones, acting on the same receptor [131].

LysMCre-IL-1β KO model

Many cell types are able to produce IL-1β, i.a. leukocytes of the myeloid lineage like macrophages and monocytes [132, 133]. If myeloid-cell derived IL-1β plays a role in GDM was tested with a mouse strain where myeloid lineage cells are IL-1β

deficient: The LysMCre-IL-1β KO strain. High-fat diet-fed LysMCre-IL-1β KO mice had improved glucose tolerance compared to their littermate controls, independent of pregnancy.

Interestingly, the plasma insulin levels of the LysMCre-IL-1β KO mice tended to be lower than that of wild type mice, hinting to a decrease of insulin resistance and not to an increase of β-cell function. These data are still preliminary and have to be confirmed in independent cohorts with the same settings as in the wild-type GDM model. Nevertheless, our results suggest that that the lack of myeloid-cell derived IL-1β protects from glucose intolerance during pregnancy.

Conclusion

In this study we show that treating mice with GDM, and healthy pregnant mice with an anti-inflammatory therapy improves their glucose tolerance. Although our results do not allow final conclusions, we propose the following mechanism:

During pregnancy, immune cells of the myeloid lineage in the decidua, close to the maternal-fetal interface, produce IL-1β. The cytokine acts on the placenta, where it stimulates the production of steroid hormones and possibly the production of other placental factors which induce insulin resistance in the maternal body and have potentially influence on the pancreatic insulin response.

In GDM, the effect of IL-1β is enhanced in the course of the proinflammatory changes in this disease. This supports the concept of GDM as an inflammatory disease.

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Bibliography

1. Ben-Haroush, A., Y. Yogev, and M. Hod, *Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes.* Diabet Med, 2004. **21**(2): p. 103-13.
2. Melchior, H., D. Kurch-Bek, and M. Mund, *The Prevalence of Gestational Diabetes.* Dtsch Arztebl Int, 2017. **114**(24): p. 412-418.
3. Egan, A.M., et al., *Epidemiology of gestational diabetes mellitus according to IADPSG/WHO 2013 criteria among obese pregnant women in Europe.* Diabetologia, 2017. **60**(10): p. 1913-1921.
4. Weissgerber, T.L. and L.M. Mudd, *Preeclampsia and diabetes.* Curr Diab Rep, 2015. **15**(3): p. 9.
5. Group, H.S.C.R., et al., *Hyperglycemia and adverse pregnancy outcomes.* N Engl J Med, 2008. **358**(19): p. 1991-2002.
6. Hadarits, O., et al., *Increased Proportion of Hematopoietic Stem and Progenitor Cell Population in Cord Blood of Neonates Born to Mothers with Gestational Diabetes Mellitus.* Stem Cells Dev, 2016. **25**(1): p. 13-7.
7. Banerjee, S., et al., *Lower whole blood ionized magnesium concentrations in hypocalcemic infants of gestational diabetic mothers.* Magnes Res, 2003. **16**(2): p. 127-30.
8. Mortier, I., et al., *Is gestational diabetes an independent risk factor of neonatal severe respiratory distress syndrome after 34 weeks of gestation? A prospective study.* Arch Gynecol Obstet, 2017. **296**(6): p. 1071-1077.
9. Kim, C., K.M. Newton, and R.H. Knopp, *Gestational diabetes and the incidence of type 2 diabetes: a systematic review.* Diabetes Care, 2002. **25**(10): p. 1862-8.
10. Burlina, S., M.G. Dalfra, and A. Lapolla, *Short- and long-term consequences for offspring exposed to maternal diabetes: a review.* J Matern Fetal Neonatal Med, 2017: p. 1-8.
11. Martinez M, W.X., Mora-Marquez J, Page KA, Buchanan T, Xiang AH. *Intrauterine exposure to maternal diabetes and risk of childhood asthma*. in *77th Annual American Diabetes Association (ADA) Scientific Sessions*. 2017. San Diego.
12. Kumar, R., et al., *Gestational diabetes, atopic dermatitis, and allergen sensitization in early childhood.* J Allergy Clin Immunol, 2009. **124**(5): p. 1031-8 e1-4.
13. Homko, C.J., et al., *Fuel metabolism during pregnancy.* Semin Reprod Endocrinol, 1999. **17**(2): p. 119-25.
14. Hay, W.W., Jr., *Placental-fetal glucose exchange and fetal glucose metabolism.* Trans Am Clin Climatol Assoc, 2006. **117**: p. 321-39; discussion 339-40.
15. Illsley, N.P., *Glucose transporters in the human placenta.* Placenta, 2000. **21**(1): p. 14-22.

16. Kevorkova, O., M. Ethier-Chiasson, and J. Lafond, *Differential expression of glucose transporters in rabbit placenta: effect of hypercholesterolemia in dams.* Biol Reprod, 2007. **76**(3): p. 487-95.
17. Li, H., et al., *High glucose levels down-regulate glucose transporter expression that correlates with increased oxidative stress in placental trophoblast cells in vitro.* J Soc Gynecol Investig, 2004. **11**(2): p. 75-81.
18. Barash, V., A. Gutman, and E. Shafrir, *Mechanism of placental glycogen deposition in diabetes in the rat.* Diabetologia, 1983. **24**(1): p. 63-8.
19. Blows, J.M., et al., *The structure of placental glycogen.* Placenta, 1988. **9**(5): p. 493-500.
20. Hay, W.W., Jr., *Energy and substrate requirements of the placenta and fetus.* Proc Nutr Soc, 1991. **50**(2): p. 321-36.
21. Kalhan, S.C., et al., *Glucose production in pregnant women at term gestation. Sources of glucose for human fetus.* J Clin Invest, 1979. **63**(3): p. 388-94.
22. Catalano, P.M., et al., *Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women.* Am J Obstet Gynecol, 1992. **167**(4 Pt 1): p. 913-9.
23. Catalano, P.M., et al., *Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus.* Am J Obstet Gynecol, 1999. **180**(4): p. 903-16.
24. Sonagra, A.D., et al., *Normal pregnancy- a state of insulin resistance.* J Clin Diagn Res, 2014. **8**(11): p. CC01-3.
25. Musial, B., et al., *Proximity to Delivery Alters Insulin Sensitivity and Glucose Metabolism in Pregnant Mice.* Diabetes, 2016. **65**(4): p. 851-60.
26. Buchanan, T.A., B.E. Metzger, and N. Freinkel, *Accelerated starvation in late pregnancy: a comparison between obese women with and without gestational diabetes mellitus.* Am J Obstet Gynecol, 1990. **162**(4): p. 1015-20.
27. Herrera, E., *Lipid metabolism in pregnancy and its consequences in the fetus and newborn.* Endocrine, 2002. **19**(1): p. 43-55.
28. Newbern, D. and M. Freemark, *Placental hormones and the control of maternal metabolism and fetal growth.* Curr Opin Endocrinol Diabetes Obes, 2011. **18**(6): p. 409-16.
29. Lappas, M., et al., *Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies.* J Endocrinol, 2005. **186**(3): p. 457-65.
30. Pala, H.G., et al., *Adiponectin levels in gestational diabetes mellitus and in pregnant women without glucose intolerance.* Adv Clin Exp Med, 2015. **24**(1): p. 85-92.
31. Catalano, P.M., *Trying to understand gestational diabetes.* Diabet Med, 2014. **31**(3): p. 273-81.
32. Rieck, S. and K.H. Kaestner, *Expansion of beta-cell mass in response to pregnancy.* Trends Endocrinol Metab, 2010. **21**(3): p. 151-8.

33. Zhang, H., et al., *Gestational diabetes mellitus resulting from impaired beta-cell compensation in the absence of FoxM1, a novel downstream effector of placental lactogen.* Diabetes, 2010. **59**(1): p. 143-52.
34. Kim, H., et al., *Serotonin regulates pancreatic beta cell mass during pregnancy.* Nat Med, 2010. **16**(7): p. 804-8.
35. Ernst, S., et al., *Mechanisms in the adaptation of maternal beta-cells during pregnancy.* Diabetes Manag (Lond), 2011. **1**(2): p. 239-248.
36. Qiao, L., et al., *Adiponectin Deficiency Impairs Maternal Metabolic Adaptation to Pregnancy in Mice.* Diabetes, 2017. **66**(5): p. 1126-1135.
37. Karnik, S.K., et al., *Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus.* Science, 2007. **318**(5851): p. 806-9.
38. Nielsen, J.H., *Beta cell adaptation in pregnancy: a tribute to Claes Hellerstrom.* Ups J Med Sci, 2016. **121**(2): p. 151-4.
39. Butler, A.E., et al., *Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy.* Diabetologia, 2010. **53**(10): p. 2167-76.
40. Warning, J.C., S.A. McCracken, and J.M. Morris, *A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system.* Reproduction, 2011. **141**(6): p. 715-24.
41. Faas, M.M., F. Spaans, and P. De Vos, *Monocytes and macrophages in pregnancy and pre-eclampsia.* Front Immunol, 2014. **5**: p. 298.
42. Sanci, M., et al., *Reference values for maternal total and differential leukocyte counts in different trimesters of pregnancy and the initial postpartum period in western Turkey.* J Obstet Gynaecol, 2017. **37**(5): p. 571-575.
43. Carter, A.M., *Animal models of human placentation--a review.* Placenta, 2007. **28 Suppl A**: p. S41-7.
44. Liu, S., et al., *The role of decidual immune cells on human pregnancy.* J Reprod Immunol, 2017. **124**: p. 44-53.
45. Madeja, Z., et al., *Paternal MHC expression on mouse trophoblast affects uterine vascularization and fetal growth.* Proc Natl Acad Sci U S A, 2011. **108**(10): p. 4012-7.
46. Faas, M.M. and P. de Vos, *Uterine NK cells and macrophages in pregnancy.* Placenta, 2017. **56**: p. 44-52.
47. Rajagopalan, S., et al., *Activation of NK cells by an endocytosed receptor for soluble HLA-G.* PLoS Biol, 2006. **4**(1): p. e9.
48. Habbeddine, M., et al., *Leukocyte population dynamics and detection of IL-9 as a major cytokine at the mouse fetal-maternal interface.* PLoS One, 2014. **9**(9): p. e107267.
49. Bulmer, J.N., P.J. Williams, and G.E. Lash, *Immune cells in the placental bed.* Int J Dev Biol, 2010. **54**(2-3): p. 281-94.
50. Houser, B.L., et al., *Two unique human decidual macrophage populations.* J Immunol, 2011. **186**(4): p. 2633-42.
51. Collins, M.K., C.S. Tay, and A. Erlebacher, *Dendritic cell entrapment within the pregnant uterus inhibits immune surveillance of the maternal/fetal interface in mice.* J Clin Invest, 2009. **119**(7): p. 2062-73.
52. Amsalem, H., et al., *Identification of a novel neutrophil population: proangiogenic granulocytes in second-trimester human decidua.* J Immunol, 2014. **193**(6): p. 3070-9.

53. Croxatto, D., et al., *Group 3 innate lymphoid cells regulate neutrophil migration and function in human decidua.* Mucosal Immunol, 2016. **9**(6): p. 1372-1383.
54. Yeung, E.H., et al., *Longitudinal study of insulin resistance and sex hormones over the menstrual cycle: the BioCycle Study.* J Clin Endocrinol Metab, 2010. **95**(12): p. 5435-42.
55. Diamanti-Kandarakis, E. and A. Dunaif, *Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications.* Endocr Rev, 2012. **33**(6): p. 981-1030.
56. Fan, W., et al., *Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion.* Diabetes, 2005. **54**(4): p. 1000-8.
57. Camporez, J.P., et al., *Cellular mechanism by which estradiol protects female ovariectomized mice from high-fat diet-induced hepatic and muscle insulin resistance.* Endocrinology, 2013. **154**(3): p. 1021-8.
58. Kapoor, D., et al., *Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes.* Eur J Endocrinol, 2006. **154**(6): p. 899-906.
59. Fabrykant, M., R.S. Jackson, and B.I. Ashe, *Cushing's syndrome: failure to demonstrate diminished peripheral glucose uptake and insulin resistance.* Metabolism, 1957. **6**(2): p. 116-26.
60. Geer, E.B., J. Islam, and C. Buettner, *Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism.* Endocrinol Metab Clin North Am, 2014. **43**(1): p. 75-102.
61. Kolter, T. *Corticosteron*. RÖMPP [web encyclopedia of chemistry] 2010 august 2010; Available from: https://roempp.thieme.de/roempp4.0/do/data/RD-03-02607.
62. van Donkelaar, E.L., et al., *Long-term corticosterone exposure decreases insulin sensitivity and induces depressive-like behaviour in the C57BL/6NCrl mouse.* PLoS One, 2014. **9**(10): p. e106960.
63. Hofmann, A., et al., *Elevated Steroid Hormone Production in the db/db Mouse Model of Obesity and Type 2 Diabetes.* Horm Metab Res, 2017. **49**(1): p. 43-49.
64. Seki, H., et al., *The regulation of progesterone and hCG production from placental cells by interleukin-1beta.* Biochim Biophys Acta, 1997. **1336**(2): p. 342-8.
65. Baratta, M., et al., *Effects of interleukin-1 beta fragment (163-171) on progesterone and estradiol-17 beta release by bovine granulosa cells from different size follicles.* Regul Pept, 1996. **67**(3): p. 187-94.
66. Fleshner, M., et al., *Interleukin-1 beta induced corticosterone elevation and hypothalamic NE depletion is vagally mediated.* Brain Res Bull, 1995. **37**(6): p. 605-10.
67. Andreis, P.G., et al., *Interleukin-1 beta enhances corticosterone secretion by acting directly on the rat adrenal gland.* Endocrinology, 1991. **129**(1): p. 53-7.
68. van der Meer, M.J., et al., *Acute stimulation of the hypothalamic-pituitary-adrenal axis by IL-1 beta, TNF alpha and IL-6: a dose response study.* J Endocrinol Invest, 1996. **19**(3): p. 175-82.

69. Beach, J.E., et al., *Rapid release of multiple hormones from rat pituitaries perifused with recombinant interleukin-1.* Life Sci, 1989. **44**(1): p. 1-7.
70. Urwyler, S.A., et al., *Interleukin-1 Antagonism Decreases Cortisol Levels in Obese Individuals.* J Clin Endocrinol Metab, 2017. **102**(5): p. 1712-1718.
71. Buchanan, T.A., *Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes.* J Clin Endocrinol Metab, 2001. **86**(3): p. 989-93.
72. Karnik, S.K., et al., *Menin regulates pancreatic islet growth by promoting histone methylation and expression of genes encoding p27Kip1 and p18INK4c.* Proc Natl Acad Sci U S A, 2005. **102**(41): p. 14659-64.
73. Barbour, L.A., et al., *Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes.* Diabetes Care, 2007. **30 Suppl 2**: p. S112-9.
74. Challier, J.C., et al., *Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta.* Placenta, 2008. **29**(3): p. 274-81.
75. Pantham, P., I.L. Aye, and T.L. Powell, *Inflammation in maternal obesity and gestational diabetes mellitus.* Placenta, 2015. **36**(7): p. 709-15.
76. Stoikou, M., et al., *Gestational Diabetes Mellitus Is Associated with Altered Neutrophil Activity.* Front Immunol, 2017. **8**: p. 702.
77. Vitoratos, N., et al., *Pre- and early post-partum adiponectin and interleukin-1beta levels in women with and without gestational diabetes.* Hormones (Athens), 2008. **7**(3): p. 230-6.
78. Christian, L.M. and K. Porter, *Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index.* Cytokine, 2014. **70**(2): p. 134-40.
79. Lappas, M., *Activation of inflammasomes in adipose tissue of women with gestational diabetes.* Mol Cell Endocrinol, 2014. **382**(1): p. 74-83.
80. Liong, S. and M. Lappas, *Endoplasmic reticulum stress is increased in adipose tissue of women with gestational diabetes.* PLoS One, 2015. **10**(4): p. e0122633.
81. Aagaard, K., et al., *The placenta harbors a unique microbiome.* Sci Transl Med, 2014. **6**(237): p. 237ra65.
82. Bassols, J., et al., *Gestational diabetes is associated with changes in placental microbiota and microbiome.* Pediatr Res, 2016. **80**(6): p. 777-784.
83. Antony, K.M., et al., *The preterm placental microbiome varies in association with excess maternal gestational weight gain.* Am J Obstet Gynecol, 2015. **212**(5): p. 653 e1-16.
84. Kuang, Y.S., et al., *Connections between the human gut microbiome and gestational diabetes mellitus.* Gigascience, 2017. **6**(8): p. 1-12.
85. Dinarello, C.A., L. Renfer, and S.M. Wolff, *Human leukocytic pyrogen: purification and development of a radioimmunoassay.* Proc Natl Acad Sci U S A, 1977. **74**(10): p. 4624-7.
86. Gery, I., R.K. Gershon, and B.H. Waksman, *Potentiation of the T-lymphocyte response to mitogens. I. The responding cell.* J Exp Med, 1972. **136**(1): p. 128-42.

87. Garlanda, C., C.A. Dinarello, and A. Mantovani, *The interleukin-1 family: back to the future.* Immunity, 2013. **39**(6): p. 1003-18.
88. Dror, E., et al., *Postprandial macrophage-derived IL-1beta stimulates insulin, and both synergistically promote glucose disposal and inflammation.* Nat Immunol, 2017. **18**(3): p. 283-292.
89. Marette, A., *Mediators of cytokine-induced insulin resistance in obesity and other inflammatory settings.* Curr Opin Clin Nutr Metab Care, 2002. **5**(4): p. 377-83.
90. Gao, D., et al., *Interleukin-1beta mediates macrophage-induced impairment of insulin signaling in human primary adipocytes.* Am J Physiol Endocrinol Metab, 2014. **307**(3): p. E289-304.
91. Bing, C., *Is interleukin-1beta a culprit in macrophage-adipocyte crosstalk in obesity?* Adipocyte, 2015. **4**(2): p. 149-52.
92. Masters, S.L., et al., *Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes.* Nat Immunol, 2010. **11**(10): p. 897-904.
93. Maedler, K., et al., *Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets.* J Clin Invest, 2002. **110**(6): p. 851-60.
94. Nordmann, T.M., et al., *The Role of Inflammation in beta-cell Dedifferentiation.* Sci Rep, 2017. **7**(1): p. 6285.
95. Boni-Schnetzler, M., et al., *Increased interleukin (IL)-1beta messenger ribonucleic acid expression in beta -cells of individuals with type 2 diabetes and regulation of IL-1beta in human islets by glucose and autostimulation.* J Clin Endocrinol Metab, 2008. **93**(10): p. 4065-74.
96. Boni-Schnetzler, M., et al., *Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor I.* Endocrinology, 2009. **150**(12): p. 5218-29.
97. Herder, C., et al., *The IL-1 Pathway in Type 2 Diabetes and Cardiovascular Complications.* Trends Endocrinol Metab, 2015. **26**(10): p. 551-63.
98. Donath, M.Y., *Multiple benefits of targeting inflammation in the treatment of type 2 diabetes.* Diabetologia, 2016. **59**(4): p. 679-82.
99. Larsen, C.M., et al., *Interleukin-1-receptor antagonist in type 2 diabetes mellitus.* N Engl J Med, 2007. **356**(15): p. 1517-26.
100. Ridker, P.M., et al., *Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS).* Am Heart J, 2011. **162**(4): p. 597-605.
101. van Asseldonk, E.J., et al., *One week treatment with the IL-1 receptor antagonist anakinra leads to a sustained improvement in insulin sensitivity in insulin resistant patients with type 1 diabetes mellitus.* Clin Immunol, 2015. **160**(2): p. 155-62.
102. Strajhar, P., et al., *Acute Effects of Lysergic Acid Diethylamide on Circulating Steroid Levels in Healthy Subjects.* J Neuroendocrinol, 2016. **28**(3): p. 12374.
103. Sauter, N.S., et al., *Angiotensin II induces interleukin-1beta-mediated islet inflammation and beta-cell dysfunction independently of vasoconstrictive effects.* Diabetes, 2015. **64**(4): p. 1273-83.

104. Laboratories, K. *Steroid hormone biosynthesis - Mus musculus (mouse)*. Kyoto Encyclopedia of Genes and Genomes (KEGG PATHWAY) 2016; Available from: http://www.genome.jp/kegg-bin/show_pathway?org_name=mmu&mapno=00140&mapscale=&show_description=show.
105. Bowe, J.E., et al., *Metabolic phenotyping guidelines: assessing glucose homeostasis in rodent models.* J Endocrinol, 2014. **222**(3): p. G13-25.
106. Owyang, A.M., et al., *XOMA 052, an anti-IL-1{beta} monoclonal antibody, improves glucose control and {beta}-cell function in the diet-induced obesity mouse model.* Endocrinology, 2010. **151**(6): p. 2515-27.
107. Wen, H., et al., *Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling.* Nat Immunol, 2011. **12**(5): p. 408-15.
108. Ehses, J.A., et al., *Toll-like receptor 2-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet.* Diabetologia, 2010. **53**(8): p. 1795-806.
109. Winzell, M.S. and B. Ahren, *The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes.* Diabetes, 2004. **53 Suppl 3**: p. S215-9.
110. Romero, A., et al., *Relationship between fetal weight and litter size in rats: application to reproductive toxicology studies.* Reprod Toxicol, 1992. **6**(5): p. 453-6.
111. Mu, J., et al., *In vivo quantification of embryonic and placental growth during gestation in mice using micro-ultrasound.* Reprod Biol Endocrinol, 2008. **6**: p. 34.
112. Jones, H.N., et al., *High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice.* FASEB J, 2009. **23**(1): p. 271-8.
113. Whyte, J.J., et al., *Maternal diet composition alters serum steroid and free fatty acid concentrations and vaginal pH in mice.* J Endocrinol, 2007. **192**(1): p. 75-81.
114. Hohos, N.M. and M.E. Skaznik-Wikiel, *High-Fat Diet and Female Fertility.* Endocrinology, 2017. **158**(8): p. 2407-2419.
115. Palmer, N.O., et al., *Impact of obesity on male fertility, sperm function and molecular composition.* Spermatogenesis, 2012. **2**(4): p. 253-263.
116. Dinarello, C.A., *Blocking IL-1 in systemic inflammation.* J Exp Med, 2005. **201**(9): p. 1355-9.
117. Medrikova, D., et al., *Sex differences during the course of diet-induced obesity in mice: adipose tissue expandability and glycemic control.* Int J Obes (Lond), 2012. **36**(2): p. 262-72.
118. Bullwinkel, J., et al., *Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells.* J Cell Physiol, 2006. **206**(3): p. 624-35.
119. Rieck, S., et al., *The transcriptional response of the islet to pregnancy in mice.* Mol Endocrinol, 2009. **23**(10): p. 1702-12.
120. Dai, G., et al., *Maternal hepatic growth response to pregnancy in the mouse.* Exp Biol Med (Maywood), 2011. **236**(11): p. 1322-32.
121. Bustamante, J.J., G. Dai, and M.J. Soares, *Pregnancy and lactation modulate maternal splenic growth and development of the erythroid lineage in the rat and mouse.* Reprod Fertil Dev, 2008. **20**(2): p. 303-10.

122. Zhang, L., et al., *The inflammatory changes of adipose tissue in late pregnant mice.* J Mol Endocrinol, 2011. **47**(2): p. 157-65.
123. Sivan, E., et al., *Effect of insulin on fat metabolism during and after normal pregnancy.* Diabetes, 1999. **48**(4): p. 834-8.
124. Barlow, S.M., P.J. Morrison, and F.M. Sullivan, *Plasma corticosterone levels during pregnancy in the mouse.* Br J Pharmacol, 1973. **48**(2): p. 346P.
125. Malassine, A., J.L. Frendo, and D. Evain-Brion, *A comparison of placental development and endocrine functions between the human and mouse model.* Hum Reprod Update, 2003. **9**(6): p. 531-9.
126. Brain, P.F. and N.W. Nowell, *Adrenal function in pregnant and lactating mice.* J Endocrinol, 1970. **48**(1): p. 17-8.
127. Barlow, S.M., P.J. Morrison, and F.M. Sullivan, *Plasma corticosterone levels during pregnancy in the mouse: the relative contributions of the adrenal glands and foeto-placental units.* J Endocrinol, 1974. **60**(3): p. 473-83.
128. Burton, P.J. and B.J. Waddell, *11 beta-Hydroxysteroid dehydrogenase in the rat placenta: developmental changes and the effects of altered glucocorticoid exposure.* J Endocrinol, 1994. **143**(3): p. 505-13.
129. Wang, F., et al., *Preeclampsia induced by cadmium in rats is related to abnormal local glucocorticoid synthesis in placenta.* Reprod Biol Endocrinol, 2014. **12**: p. 77.
130. Vinson, G.P., *The mislabelling of deoxycorticosterone: making sense of corticosteroid structure and function.* J Endocrinol, 2011. **211**(1): p. 3-16.
131. Handwerger, S. and M. Freemark, *The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development.* J Pediatr Endocrinol Metab, 2000. **13**(4): p. 343-56.
132. Arango Duque, G. and A. Descoteaux, *Macrophage cytokines: involvement in immunity and infectious diseases.* Front Immunol, 2014. **5**: p. 491.
133. Schutte, R.J., A. Parisi-Amon, and W.M. Reichert, *Cytokine profiling using monocytes/macrophages cultured on common biomaterials with a range of surface chemistries.* J Biomed Mater Res A, 2009. **88**(1): p. 128-39.

Curriculum Vitae

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Born February 11, 1987 in Bielefeld, Germany
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Education

January 2016:	conferral of a medical doctorate of the University of Basel
Since November 2013:	MD-PhD in the Department of Biomedicine, University Hospital and University of Basel, under the direction of Marc Y. Donath; topic: "The role of interleukin-1β in glucose metabolism during pregnancy and in gestational diabetes mellitus"
2007-2013:	medical school at the university of Basel
2006- 2007:	chemistry studies at the Rheinische Friedrich-Wilhelm Universität in Bonn, Germany
2005-2006:	medical school at the university Louis-Pasteur, Strasbourg, France
1997-2005:	German-French high school in Freiburg i.Br., Germany; advanced courses in mathematics and natural sciences

Languages

German:	Native Language
English:	good knowledge
French:	good knowledge

Clinical Internships:

2012-2013:	Practical year: 2 months internal medicine, University Hospital Basel 2 months internal medicine, Changi General Hospital, Singapore 2 months general surgery, Hôpital du Jura, Delémont 1 month neurology, University Hospital Basel 1 month nephrology University Hospital Basel

Commitment:

Currently:	Board member of the Swiss MD-PhD Association Vice chair of the European MD-PhD Association
During Medical school:	4 years co-direction of a summer camp for children with muscular diseases
During high school:	member of the school first-aid service Direction of the pupil's library